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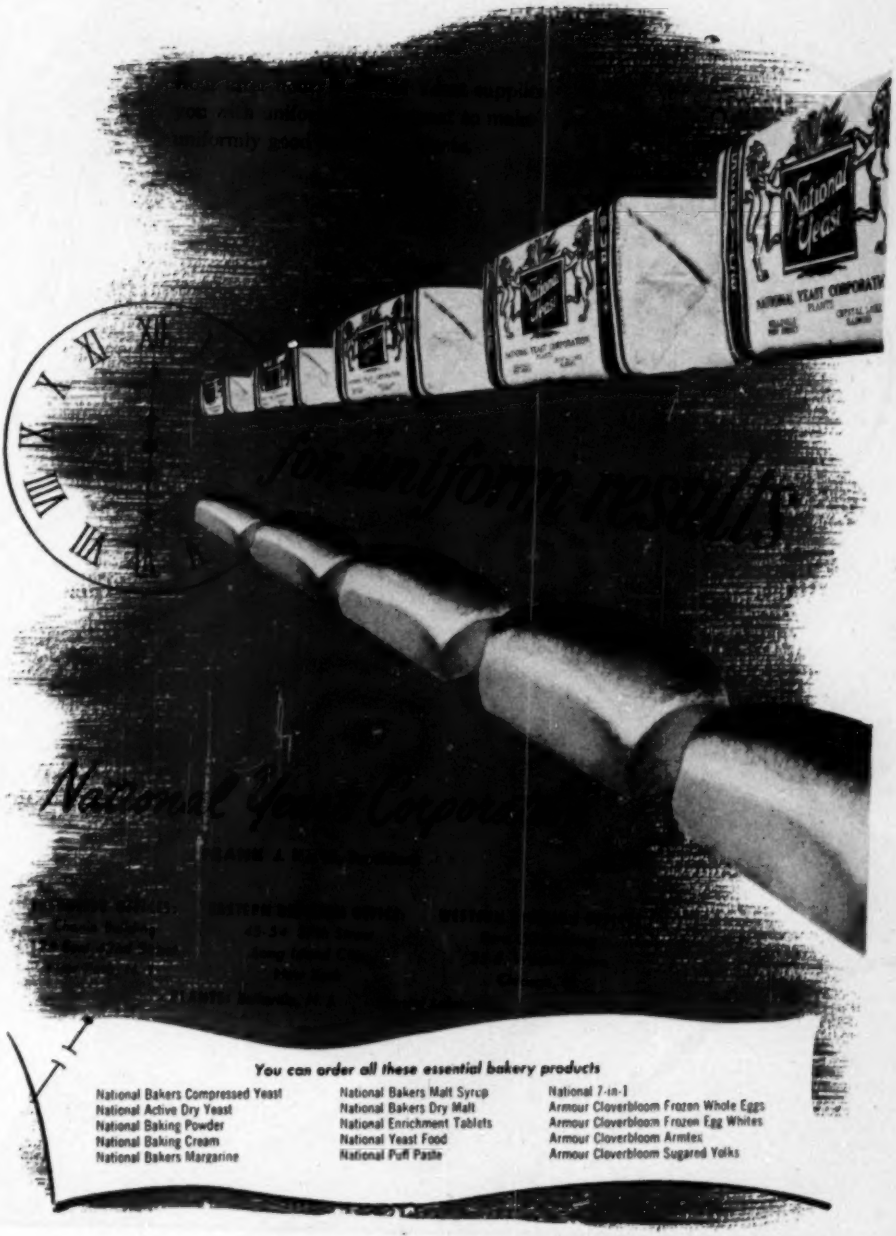

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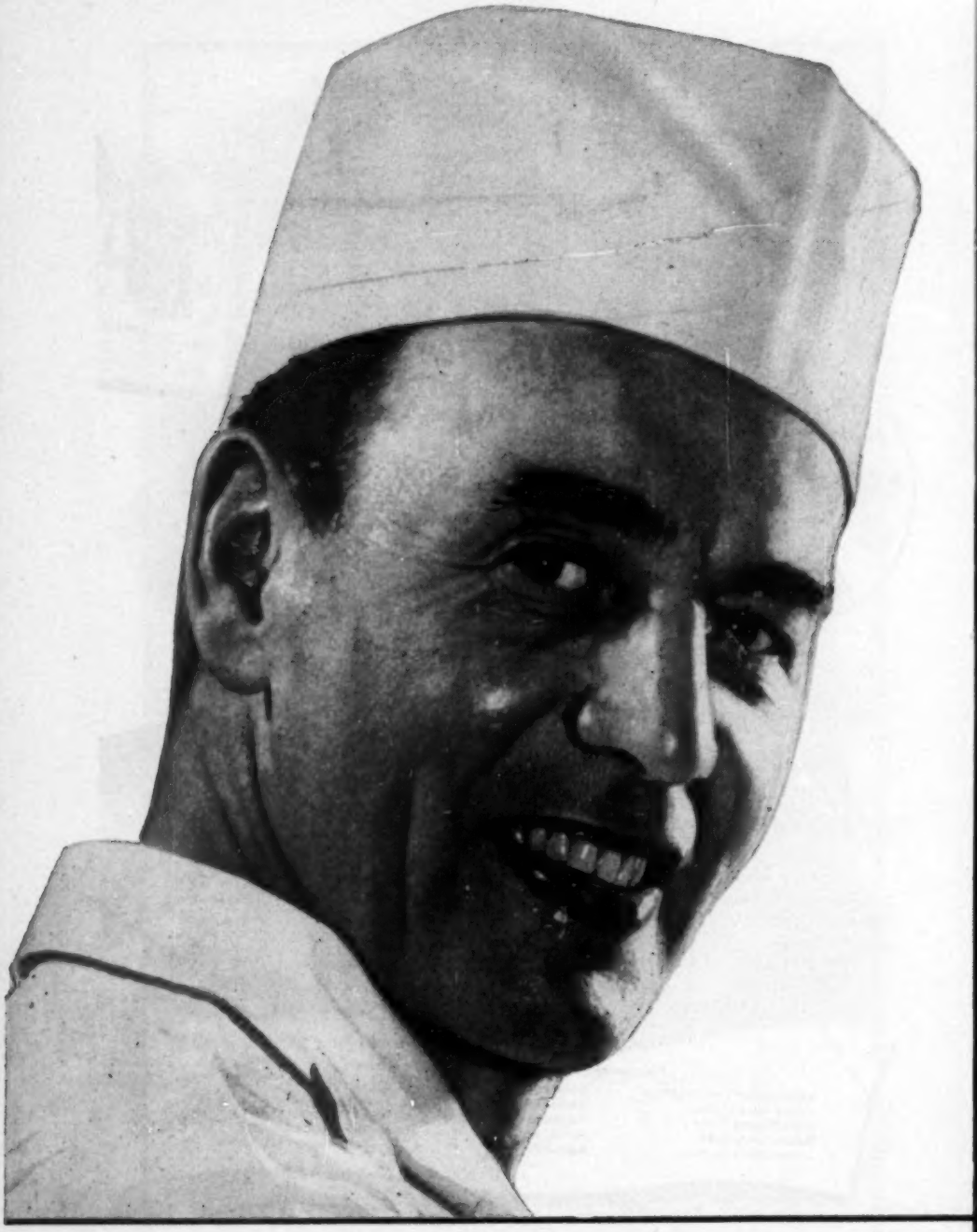

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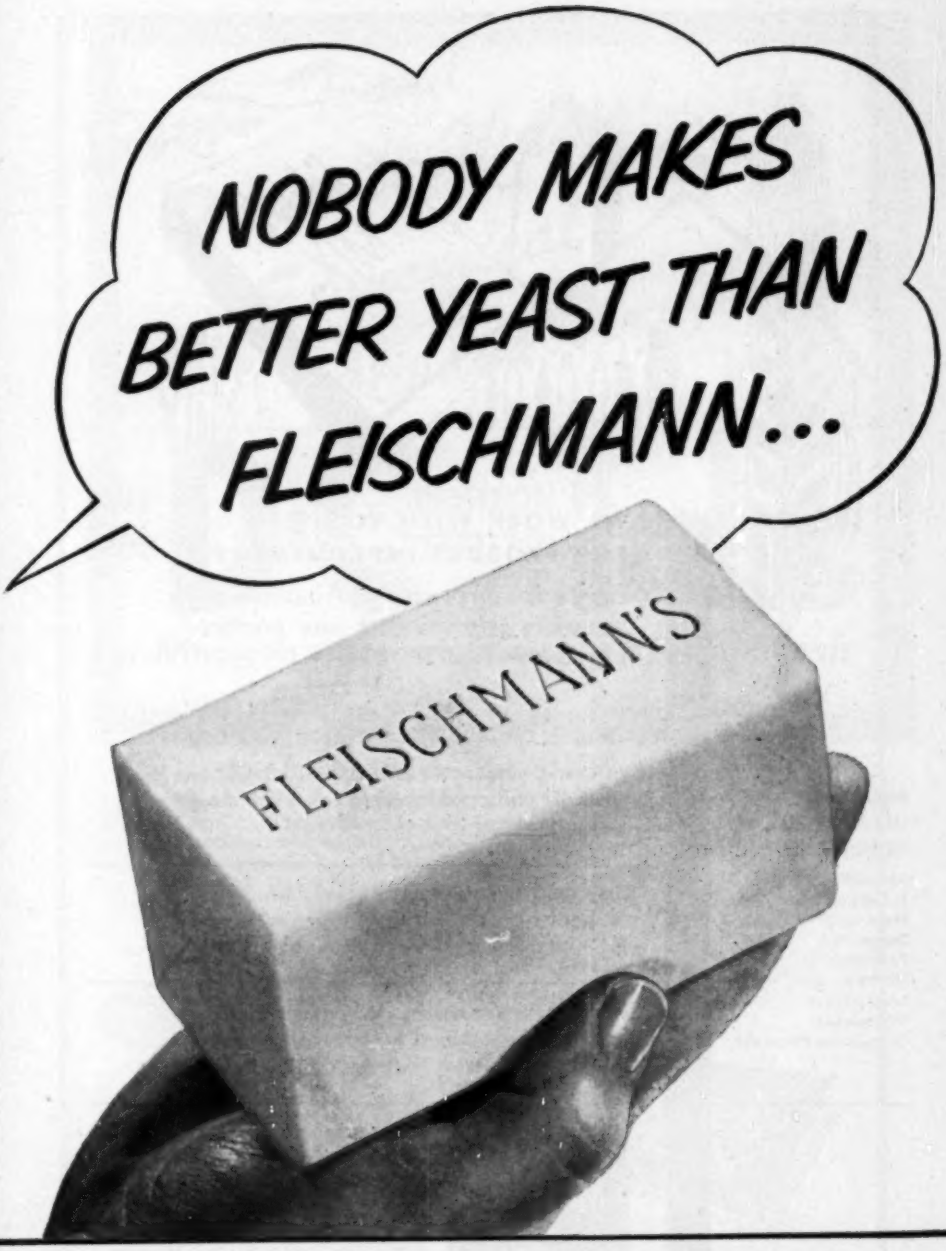

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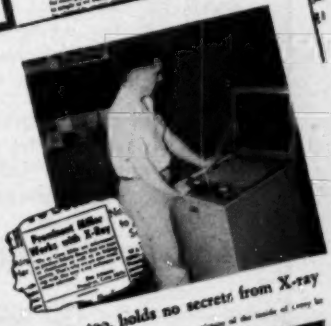

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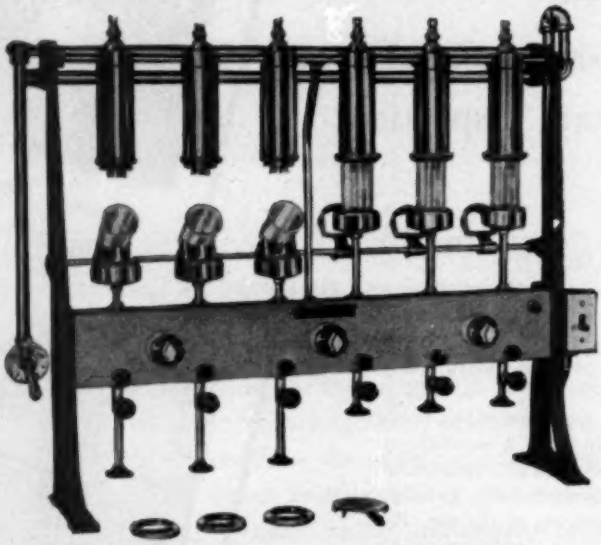

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CEREAL CHEMISTRY

PREPARATION AND SOME PROPERTIES OF HEMICELLULOSES FROM CORN HULLS[1, 2]

M. J. WOLF, M. M. MACMASTERS, JOHN A. CANNON,[3] E. C. ROSEWALL, and C. E. RIST[4]

ABSTRACT

Hemicelluloses in yields of 41 to 46% were extracted by laboratory methods from corn pericarp and commercial corn bran (dry-milled). Yields varied from 32 to 40% from commercial coarse fiber (wet-milled). In the preferred treatment, the hemicelluloses were solubilized by boiling the ground hulls at pH 10.5 to 11.5 in sodium hydroxide solution, and then extracting repeatedly with hot water. The products showed a negative rotation ($[\alpha]_D^{25} - 81$, $c = 1.0$, in water). Analysis by qualitative and quantitative paper chromatography showed that the hemicelluloses from corn pericarp were composed of 48% xylose, 35% arabinose, and 7% galactose. Uronic acid was not identified by paper chromatography in the products of hydrolysis; however, the hemicelluloses gave a positive naphthoresorcinol test and the presence of free carbonyl was indicated by infra-red absorption. From 7 to 12% of uronic acid was found, by titration, in various preparations. The hemicelluloses were highly soluble in water to form homogeneous solutions varying from light straw to brown in color. The pH of solutions of the hemicelluloses varied from about 3 for the free acid to about 7.3 for the sodium salt. The solution viscosity (measured in 0.5 to 5% concentration) was considerably higher than that of gum arabic, but lower than that of some vegetable gums such as karaya and tragacanth. The relative ease of extraction of the hemicelluloses from cheap industrial by-products and the favorable physical properties of the aqueous solutions suggest that these polysaccharides from corn hulls may prove useful as thickeners and adhesives.

The corn kernel has an outer, fibrous covering, the hull, composed of the pericarp, seed coat, and hilar layer (20). In most varieties, the hull comprises from 5 to 6% of the weight of the kernel (7).

Corn hull is a by-product of the industrial processing of corn by

[1] Manuscript received May 25, 1953.
[2] Presented at the Annual Meeting of the American Association of Cereal Chemists, Buffalo, New York, May 24-28, 1953.
[3] Present address: Pabst Brewing Company, Peoria Heights, Illinois.
[4] Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U.S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

both the wet- and dry-milling methods. The coarse fiber obtained by the wet milling of corn is composed chiefly of hull. This fraction is mixed with other by-product fractions of the kernel for sale as gluten feed. Similarly, in dry milling, the bran fraction, which also is largely hull, is mixed with other by-product fractions and sold as hominy feed. In both the wet- and dry-milling industries, the mixing is done at the processing plant and no hull is marketed as such.

The other feed constituents contain from three to five times as much protein as is in the hull. If the industrial use of corn protein, as for example to produce zein fibers, is considerably expanded, part of the output of hull must be used elsewhere than in feed in order to maintain the established protein content of the latter.

Corn hull is available in processing plants in relatively large quantities. On the basis of the reported grind (3), about 196,600 tons of coarse fiber were produced in 1949, and probably about one-half that amount of bran. Perhaps the chief reason why industrial use of these fractions has not been made is that the composition of corn hull has been only cursorily studied.

Microscopic examination has shown (20) that the pericarp, which constitutes most of the hull, is made up largely of thick-walled cells nearly devoid of contents; hence polysaccharides, of which cell walls are almost entirely composed, should account for most of the dry weight of the pericarp tissue. Schulze (17) reported the pentosan content of corn hull to be 43.37%; he identified xylose and galactose as constituents of the pentosan. Porst (16) found 48.62% pentosan in corn hull; he reported 6.04% methylpentosans from the same source. From determinations of furfural yield, Hooper (10) estimated the pentosan content of the hull to be about 42 to 53%. She called attention to the high percentage of hemicelluloses in the hull, but did not isolate these constituents.

Hemicelluloses are common plant polysaccharides; often a large proportion of the cell-wall material consists of hemicelluloses. Some hemicelluloses may perhaps be removed by washing with water. In general, however, the hemicelluloses which occur as constituents of the cell walls are defined as those polysaccharides which are not extractable with water but are extractable with cold or hot alkali solutions. The hemicelluloses are readily hydrolyzed by hot, dilute mineral acids to yield sugars and usually a sugar acid.

Hemicelluloses, like other polysaccharides such as starches, dextrins, and vegetable gums, have properties which render them industrially useful. A crude hemicellulosic product from wood is said to be in commercial use as a binder (2). Hemicelluloses serve as natural ad-

hesives in paper (8). The adhesive and thickening properties of corn-hull hemicelluloses would doubtless find numerous applications if they could be made industrially available at an attractive price.

The present study was undertaken to develop methods for the separation of hemicelluloses from corn hull, and to investigate some of the physical and chemical properties of the hemicelluloses so separated. It was considered possible that information might be obtained on the basis of which a by-product from corn hulls might be developed for use as a thickener, conditioner, and size.

Preparation of Material for Extraction

Pericarp was separated in the laboratory from Iowa 306 variety hybrid dent corn, harvested in 1947 at Ames, Iowa. The kernels were soaked in distilled water at room temperature for from 0.5 to 1 hour, and the wet grain was beaten for about 1 minute in a blendor equipped with padded blades. This treatment loosened the pericarp, which was then separated from germ and endosperm fragments by wet sieving and air aspiration. The sample was finally hand-picked to remove tip caps and pieces of pericarp that had large fragments of endosperm adhering to them. The material so prepared contained less than 2% starch and about 50% pentosans (Table I).

TABLE I

PARTIAL ANALYSIS OF COARSE FIBER, BRAN, AND LABORATORY-PREPARED PERICARP (MOISTURE-FREE BASIS)

Constituent	Coarse Fiber, Unwashed			Bran		Laboratory-Prepared Pericarp
	Sample 1	Sample 2	Sample 3	Washed	Unwashed	
	%	%	%	%	%	%
Ash	0.58	0.56	0.23	0.93	1.36	0.68
Protein[1]	8.1	8.1	8.1	5.4	7.8	4.7
Carbohydrate						
Pentosan	38.5	38.7	45.2	42.8	...	49.5
Starch	14.8	22.9	8.7	4.7	22.4	1.7
Unidentified	38.02	29.74	37.77	46.17	68.44	43.42

[1] N × 6.25.

Several samples of industrial coarse fiber, from corn wet-millers, and one of bran, from a dry-miller, also were used in the study. They contained more starch, from the endosperm, and less pentosans than did the laboratory-prepared pericarp (Table I). Hull with low starch content is desirable as raw material for the preparation of hemicelluloses because, when starch content is high, starch is found in the

hemicellulosic extracts where it retrogrades and precipitates. Most of the starch present in the industrial samples was in the form of free granules which could be readily removed by washing the coarse fiber or bran with water several times before grinding (Table I). All of the raw materials were ground in a hammer mill to pass a 1/32-in. screen before the extraction was started, and before analyses were made.

Extraction and Recovery Procedures

The effect of a number of extraction variables on the yield of hemicelluloses from corn hull was studied. The conditions used are summarized in Tables II and III. The average ratios of solvent to hull extracted were about 9:1 (v/w) in the first extraction, and 7:1 in subsequent extractions. Special attention was given to a comparison of results obtained by successive extractions with alkali and by pretreatment with alkali followed by water extractions.

When solubilization of the hemicelluloses was effected by a single treatment with boiling alkali solution (Table III, Runs 8, 9, 10), reagent was added during the treatment if needed to maintain the desired pH level.

For recovery of the separated hemicelluloses, the alkaline extracts were adjusted to pH 4 or slightly lower by addition of hydrochloric acid. The hemicelluloses were then precipitated from the acidic solution by addition, with constant stirring, of three volumes of 95% ethanol or of isopropanol. In one instance, the acidic solution was spray-dried for recovery of the hemicelluloses.

Analytical Methods

Starch. Ground samples were weighed before and after treatment with bacterial α-amylase. The sample was heated at 90°C. for 20 minutes to gelatinize the starch before incubation with the enzyme. The difference in weight was taken as the weight of starch present in the original sample.

Pentosans. The method of distillation was essentially that of the A.O.A.C. (4). A total of from 390 to 450 ml. of distillate was collected. Tests of the distillate with aniline acetate (12) showed that the evolution of furfural was complete before the above volumes were obtained. The distillate was redistilled to destroy hydroxymethylfurfural, as suggested by Kullgren and Tyden (13); loss of furfural in this step was about 3%.

The percentage of furfural recovered from pure xylose by this

method was constant over the sample size range of about 0.1 to 0.3 g. The size of experimental samples was therefore adjusted to yield furfural in an amount equivalent to that obtained from 0.1 to 0.3 g. of xylose. Furfural yield from xylose, after two distillations, averaged $87 \pm 3\%$ of theory (about 40 determinations). Xylose was frequently run concurrently with unknown samples to maintain a check on the method.

Since rhamnose was not detected by paper chromatography of hydrolysates of corn hull, it was assumed that methylfurfural did not interfere with the pentosan determination.

The final distillate was made up to 500 ml. with 12% hydrochloric acid, to give an acid concentration of about 6%. The furfural in this solution was estimated by the bromination method of Hughes and Acree (11). It was determined in advance that from 7 to 10 minutes were required for the addition of 2 atoms of bromine to one molecule of furfural at 0° to 1°C. In the final titration, 1.0 ml. of 0.1 *N* sodium thiosulfate is equivalent to 4.8 mg. of furfural.

The results were calculated to anhydroxylose by multiplying the weight of furfural obtained by the factor 1.58. This gave a low value for pentoses in both the hull and the separated hemicelluloses, because the factor does not take into account the relatively low conversion of arabinose to furfural, which was found in the present study to be about 81% of theoretical.

Nitrogen. Nitrogen was determined by the Kjeldahl-Wilfarth-Gunning method (4) with Winkler's modification (19).

Ash. Ash was determined by ignition of the sample in platinum crucibles to constant weight at 550°C. in a muffle furnace.

Equivalent Weight of Hemicelluloses. The average equivalent weight of the hemicelluloses was determined on an especially prepared sample. The sample was de-ashed by cation exchange between the solid phase and an acid-alcohol solution. The hemicelluloses were stirred for a few minutes in 80% ethanol which was 0.1 *N* with respect to hydrochloric acid. The alcoholic solution was centrifuged off. After three such treatments, the hemicelluloses, now in the free-acid condition, were washed repeatedly with 80% ethanol until the washings were free of chlorides and the pH had reached a constant level. The hemicelluloses were dehydrated with absolute ethanol; the ethanol was removed over anhydrous calcium chloride in a vacuum desiccator. From 0.7 to 1.0 g. of hemicelluloses was dissolved in 50 ml. of carbon-dioxide-free distilled water, and the solution was titrated potentiometrically with approximately 0.05 *N* sodium hydroxide solution. The equivalent weight was calculated by the equation:

$$\text{Equivalent weight} = \frac{\text{g. of hemicelluloses} \times 1000}{\text{me. of sodium hydroxide}}$$

Uronic Acid Content of Hemicelluloses. To de-esterify the hemicelluloses, 5 ml. of approximately 0.5 *N* sodium hydroxide were added to the solution which had just been titrated for the determination of equivalent weight. The solution was allowed to stand for 30 minutes at room temperature in a stoppered container, and was then back-titrated potentiometrically with approximately 0.1 *N* hydrochloric acid. The sum of any alkali which had reacted with the hemicelluloses during this procedure and that which had reacted during the determination of the equivalent weight was used to calculate the anhydrouronic acid content of the hemicelluloses by the equation:

$$\text{Percent anhydrouronic acid} = \frac{\text{total me. of sodium hydroxide} \times 176 \times 100}{\text{g. of hemicelluloses} \times 1000}$$

Constituent Sugars. Qualitative and quantitative determinations of sugars in hydrolysates of hull and of separated hemicelluloses were carried out by paper chromatographic methods (21).

Solution Viscosity. The viscosity of aqueous solutions of the hemicelluloses and vegetable gums was obtained with Ostwald-Cannon-Fenske-type viscometers at 25 ± 0.1°C. The viscometers were calibrated with National Bureau of Standards oils, or with distilled water, depending upon the viscosity range of each.

The corn-hull hemicelluloses and gum arabic (U.S.P., select) dissolved readily in water at room temperature to give solutions satisfactory for viscosity determinations. Karaya (white grade, super initial, powdered), tragacanth (U.S.P. No. 1), and locust-bean gums and guar flour, however, were only partially dispersed in water at room temperature, even after vigorous stirring in a high-speed blendor. Dispersion of these gums was increased by boiling for 15 minutes or by autoclaving them in water for 30 minutes at 121°C. (15 psi).

Results and Discussion

Influence of Raw Material on Hemicellulose Yields. Treatment which the hull had previously received had an effect upon the amount of hemicelluloses which could be extracted from it. From 41 to 46% of hemicellulosic material (based on ash- and moisture-free weight of hull) could be obtained from laboratory-prepared pericarp (Table II), and from bran from dry-milled corn (Table III). Lower yields, of from 32 to 40%, could be obtained from coarse fiber from wet-milled corn (Table III). It is supposed that the steeping treatment to which

TABLE II

CONDITIONS OF EXTRACTION AND YIELDS OF HEMICELLULOSES FROM TYPICAL EXTRACTION EXPERIMENTS ON LABORATORY-PREPARED PERICARP

Conditions of Extraction	Run Number				
	1	2[1]	3[1]	4	5[2]
Wt. of pericarp, g.[3]	67	65	60	64	80
Reagent	Na_2CO_3–$NaHCO_3$ buffer	3% NaOH	0.5% NaOH	2% Na_2CO_3	Water
pH	9 (initial)	....	....	10.5 (initial)	....
Temperature, °C.	121 (15 psi)	Room temp.	94–100	Boiled	94
Time per extraction	30 min.	2, 2, 2, 5, 16 hrs.	30 min.	30 min.	1, 2, 2, 2 hrs.
No. of extractions	7	5	7	7	4
Total volume of reagent, ml.	3,500	3,400	4,400	4,200	3,060
Yield, %[4]	34	43	45	46	42

[1] Chlorinated at room temperature for 30 min. each, before and after first extraction with 100 ml. of 5.3% sodium hypochlorite solution adjusted to pH 2.0 with sulfuric acid (not included in volumes shown, since the acid solutions were discarded after the treatment).
[2] The hemicelluloses were solubilized by pretreating the pericarp with 500 ml. of 2% sodium carbonate solution (included in total volume, below) for 2 hours at 121°C. (15 psi); all of the solubilizing reagent was absorbed by the fiber on autoclaving.
[3] Moisture-free basis.
[4] Based on ash- and moisture-free weights of hemicelluloses and pericarp.

TABLE III

CONDITIONS OF EXTRACTION AND YIELDS OF HEMICELLULOSES FROM TYPICAL EXTRACTION EXPERIMENTS ON COARSE FIBER AND BRAN

Conditions	Run Number[1]							
	6	7	8	9	10	11	12	13
Solubilization	None	None						
Reagent			Na_2CO_3	NaOH	NaOH	2% Na_2CO_3[2]	2% NaOH[2]	2% Na_2CO_3[2]
pH			10.4–10.5	10.9–11.6	10.6–11.5	....	....	....
Volume, ml.			400	250	450	500	500	500
Temperature, °C.			Boiled	Boiled	Boiled	121 (15 psi)	121 (15 psi)	121 (15 psi)
Time			2 hrs.	1 hr.	1 hr.	2 hrs.	2 hrs.	2 hrs.
Extraction								
Extracting medium	2% Na_2CO_3	2% Na_2CO_3	Water	Water	Water	Water	Water	Water
pH	9.4–10.8	9.2–10.2	10.4–10.6	9.9–11.6	9.5–11.1	....	....	....
Temperature, °C.	Boiled	Boiled	Boiled	Boiled	Room temp.	94	94	94
Time per extraction	1 hr.	30 min.	1 hr.	1 hr.	1 hr.	1, 2, 2 hrs.	1, 2, 2 hrs.	1, 2, 2 hrs.
No. of extractions	5	4	4	4	5	3	3	3
Total volume, ml.	1,700	1,050	1,550	1,050	1,250	2,660	2,160	2,160
Wt. of hull, g.[3]	65	43	69	43	47	84	82	84
Yield, %[4]	33	34	33	38	39	31	32	41

[1] Runs 6 to 12 made on coarse fiber; run 13 on bran.
[2] The 500 ml. of solution were completely absorbed by the fiber on autoclaving.
[3] Moisture-free basis.
[4] Based on ash- and moisture-free weight of hemicellulose and of hull.

corn is subjected prior to wet milling may degrade a part of the hemicelluloses to yield products soluble in the steep and in the subsequent processing waters.

Effect of pH of Extraction on Hemicellulose Yields. Yields of hemicelluloses from a given raw material were proportional to the pH of the extractant up to a pH of about 11.0. No hemicelluloses could be extracted from laboratory-prepared pericarp, for example, by autoclaving it with phosphate buffer solution at pH 4.8; but at about pH 9, approximately 33% of the original material was extracted as hemicelluloses (Table II, Run 1); and at pH 10.5 or higher the yields were increased up to 46% (Table II, Runs 2, 3, 4).

The solubilization of the hemicelluloses was always associated with a pronounced swelling of the cell walls. The tissues imbibed much liquid during swelling, until the entire mass assumed a gelatinous appearance. For example, hull autoclaved in 2% sodium carbonate solution absorbed up to 7.5 times its weight of liquid. The swelling was greater the higher the pH of the solution used; and the greater the swelling, the less was the percentage of pentosan left in the hull after extraction. Nevertheless, depending upon conditions, from 9% to as much as 40% of the original pentosan content was retained in the fibrous residue (Table IV). With a given extraction procedure, pericarp tissues were exhaustively extracted in five to six steps (Fig. 1, Runs 6, 8, 10). Possibly additional polysaccharides might be extracted from the residue by resorting to stronger alkali and higher temperature. It appears that virtually complete disintegration of the cell walls by the action of strong alkali would be required to release the remaining pentosan.

Concentrations of alkali sufficient to effect disintegration of the fibrous tissues would, however, bring about degradation of the hemicelluloses. Thompson and Wise (18) noted that degradation occurred when hemicelluloses from aspen wood were heated in alkaline solution. Evidence of degradation also was obtained in the present study. On the average, from 68 to 81% of the weight of the original hull could be recovered as the sum of the separated hemicelluloses and the residue left after extraction (Table IV), depending on the source of the original hull. However, a materials balance of over 97% is obtainable if the alcohol solubles are taken into account (Table V). These materials were recovered as non-volatile organic matter from the dark-brown residue left after evaporation (at pH 7.5) of the alcoholic supernatant solution from which the hemicelluloses had been precipitated. The alcohol solubles contained little or no pentosan. Total pentosan content of hemicelluloses and residue accounted, on the average, for

TABLE IV

DISTRIBUTION OF PENTOSAN IN HEMICELLULOSES AND IN THE RESIDUE AFTER EXTRACTION OF PERICARP AND COMMERCIAL HULL

Type of Hull Preparation	Number of Runs[1]	Average Pentosan Content[2]		Average Distribution of Pentosan (% of Total Pentosan)		Average Recovery of Total	
		In Hemicelluloses	In Residue	In Hemicelluloses	In Residue	Pentosan	Weight[3]
		%	%	%	%	%	%
Pericarp, laboratory-prepared	6	77 (72–80)[4]	27 (21–37)	66 (52–73)	15 (9–23)	82 (76–89)	75 (72–78)
Coarse fiber (wet-milled)	12	73 (68–89)	37 (31–45)	56 (50–74)	25 (19–32)	81 (71–92)	68 (63–82)
Coarse fiber[5] (wet-milled)	6	61 (50–71)	33 (29–39)	57 (38–68)	30 (22–40)	88 (78–100)	74 (69–77)
Bran (dry-milled)	1	66	31	64	28	91	81

[1] Not necessarily replicates by one method.
[2] Moisture- and ash-free basis.
[3] An additional 10% of material was recovered on evaporating the alcoholic solution from which the hemicelluloses had been precipitated (average of three runs).
[4] Values in parentheses are ranges.
[5] Fiber contaminated with appreciable amounts of starch.

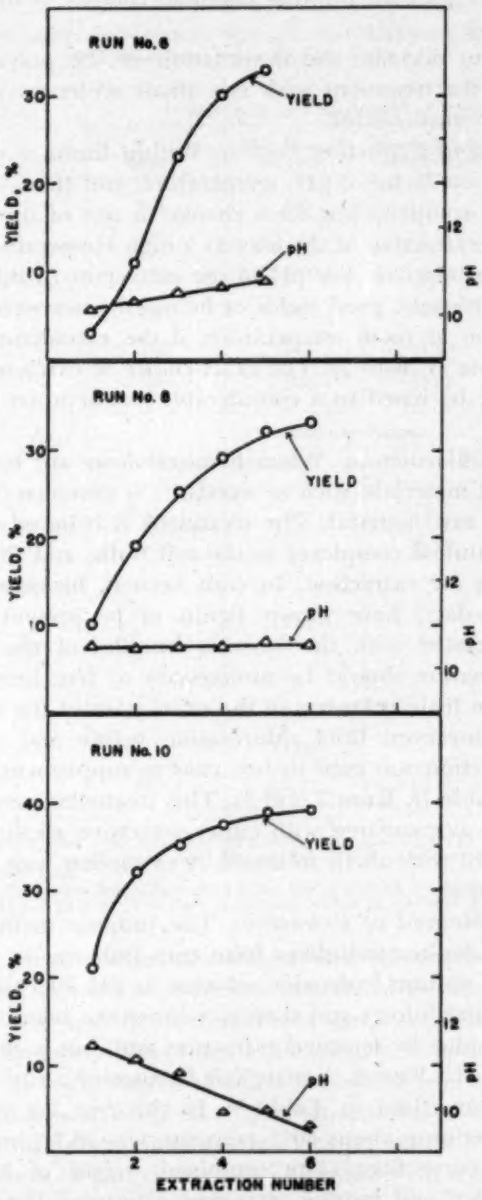

Fig. 1. pH of successive extracts and cumulative hemicellulose yields (ash- and moisture-free basis) obtained by various treatments of corn hull. All runs from Table III.

about 81 to 91% of the original pentosan content of the hull (Table IV).

Attempts to decrease the degradation of the polysaccharides by carrying out the treatment with hot alkali under an atmosphere of nitrogen were unsuccessful.

Compensating Extraction Factors. Within limits, a change in one of the extraction factors – pH, temperature, and time – was found to be capable of compensating for a change in one of the other factors. For example, extraction of the fiber at a high temperature partly compensated for a relatively low pH of the extractant (Table II, cf. Runs 4 and 5). Conversely, good yields of hemicelluloses were obtained by long extraction at room temperature, if the extractant was strongly alkaline (Table II, Run 2). The exact choice of extraction conditions may therefore be based to a considerable extent upon practical considerations.

Effect of Chlorination. When hemicelluloses are to be extracted from lignified materials, such as woods, it is customary to chlorinate the cellulosic raw material. The treatment is believed to decompose lignin-hemicellulose complexes in the cell walls, and thus to free the hemicelluloses for extraction. In corn kernels, histochemical studies (unpublished data) have shown lignin to be present only in the sclereids associated with the vascular bundles of the tip cap (20). Hence chlorination should be unnecessary to free hemicelluloses in the hull. Since little is known of the exact state of the hemicelluloses in the hull, however, brief chlorination before and after the first alkaline extraction was used in two runs to supplement the action of the alkali (Table II, Runs 2 and 3). This treatment gave no improvement in yield as compared with either successive alkaline extractions or pretreatment with alkali followed by extraction with water (Table II, Runs 4 and 5).

Preferred Method of Extraction. The simplest method found for extraction of the hemicelluloses from corn hull was to boil the fiber for 1 hour in sodium hydroxide solution at pH 10.5 to 11.5 to solubilize the hemicelluloses and then to remove the hemicelluloses from the fibrous residue by repeated extraction with hot water, as exemplified in Table III, Run 9. A materials balance of a run made by this method is summarized in Table V. In this run the yield of hemicelluloses containing about 69% pentosan was 38.3% on the basis of the washed coarse fiber. The combined weight of hemicelluloses, ethanol solubles, and residue recovered accounted for 97.4% of the washed fiber. One run (Table III, Run 10) indicated that it may be possible to remove the hemicelluloses with water at room temperature.

TABLE V

HEMICELLULOSE PREPARATION: RESULTS AND MATERIALS BALANCE OF EXTRACTION FROM COARSE FIBER[1]

	Solubilization and Extraction[2] of Hemicelluloses			
	Extract	95% Ethanol for pptn.	Hemicelluloses Precipitated	Pentosan Content
	ml.	*ml.*	*g.*	*g.*
Extraction No. 1	265	795	7.46	5.29
Extraction No. 2	225	675	4.20	2.92
Extraction No. 3	210	630	1.73	1.10
Extraction No. 4	290	870	1.23	0.75
Totals	990	2,970	14.62	10.06

	Materials Balance			
	Weight of Material		Pentosan	
	g.	*g.*	*g.*	*g.*
Coarse fiber, washed	38.22		15.17	
Total hemicelluloses precipitated in 71% ethanol		14.62		10.06
Solids soluble in 71% ethanol		7.41		
Residue		15.19		4.56
Loss		1.00		0.55
Totals	38.22	38.22	15.17	15.17

[1] 38.22 g. of coarse fiber were recovered on washing and screening 49.63 g. of commercial coarse fiber; 11.41 g. of starch, fines, and water-solubles were removed. All weights are on ash- and moisture-free basis.

[2] The hemicelluloses were solubilized during the first extraction by boiling for 1 hour with sodium hydroxide at pH 10.3 to 11.5. Only water was added in the next three extractions.

The method has obvious advantages over the conventional procedure of repeated extraction of the raw material with hot alkali, in that less reagent is used and the hemicelluloses which are extracted contain less ash. Equally good yields of hemicelluloses are obtained (Table III, cf. Runs 6 and 7 with Runs 8 through 13) by the two methods.

It has been reported (6) that pretreatment of wheat-straw holocellulose with liquid ammonia exerts a similar solubilizing effect and permits extraction of part of the hemicelluloses with water.

Recovery of Hemicelluloses from Extracts. Isopropyl alcohol was as effective as ethanol in precipitating the hemicelluloses from the extracts. Complete precipitation occurred rapidly when either alcohol

was added to solutions acidified with hydrochloric acid so that the hemicelluloses were present chiefly in the acid form. When the hemicelluloses were present as the sodium salt, a small excess of sodium chloride was added to start the precipitation with alcohol. The effect of other salts upon the precipitation has not yet been determined.

The hemicelluloses were readily recovered by spray-drying a 3% solution prepared by the preferred method. The product obtained was high in ash content (18%), however, because of salt resulting from the extraction and neutralization processes.

General Characteristics of the Hemicelluloses. The dry products were amorphous, white to light tan powders. Aqueous solutions varied from straw color to brown. The hemicelluloses were highly soluble in water, to yield homogeneous solutions that were stable over a wide pH range. Dispersion was apparently complete unless starch was carried over from the raw material. When the final product contained starch, most of it could be removed by centrifuging. The hemicellulose solutions were acidic, the pH depending upon the ash content of the sample. The pH of a solution of ash-free hemicelluloses was about 3.

Ash Content of the Hemicelluloses. The ash content of the hemicelluloses originated chiefly from the alkali used in treatment of the fiber. The original hull contained from 0.2 to 1.4% ash (Table I). Hemicelluloses extracted by the preferred method contained from 1.8 to 2.7% ash (average 2.2%). Those extracted by repeated treatments of the fiber with hot alkali contained from 2.2 to 4.5% ash.

The amount of ash carried down by the hemicelluloses when they were precipitated was greater the higher the pH of the solution. In a typical experiment, aliquots of an aqueous solution containing about 2.5% hemicelluloses and an excess of sodium chloride were adjusted to pH levels ranging from 2 to 10. The ash content of the hemicelluloses precipitated by ethanol from the solutions ranged from 0.4% at pH 2 to 4.7% at pH 10. The salt of the hemicelluloses contained from 2 to 3% of ash, expressed as sodium carbonate, depending on the uronic acid content. Higher ash contents indicated adsorption on the hemicelluloses of excess salt from solution; lower ash contents indicated that the hemicelluloses were partly in the free-acid form.

Preparations of the free hemicellulose acid containing as little as 0.04% ash were obtained by precipitating the hemicelluloses twice with ethanol from aqueous solutions 0.1 *N* with respect to hydrochloric acid.

Composition of the Hemicelluloses. Only a partial investigation has yet been made of the composition of the hemicelluloses that have been separated from corn hull by the various methods employed.

A high negative rotation was found which suggests a preponderance of β-glycosidic linkages in the molecules. Hemicelluloses from laboratory-prepared pericarp (Table II, Run 1) showed a specific rotation, $[\alpha]_D^{25} - 80$ (c = 1.0, in water), whereas that for a preparation from coarse fiber (extracted with hot 2% sodium hydroxide–2% sodium hyposulfite solution) was − 82.

Xylose, arabinose, and galactose were identified on paper chromatograms of hydrolysates. Glucose was found only in preparations from material appreciably contaminated with starch. A mixture of unidentified material of relatively low mobility and showing little or no reducing activity also was present; uronic acid was not found by chromatography. The hemicelluloses, however, gave a positive naphthoresorcinol test, and the infra-red absorption spectrum indicated the presence of free carbonyl groups. There is some evidence, therefore, that the hemicelluloses are of the polyuronide type.

Quantitative analysis by paper chromatography of a hydrolysate from hemicelluloses prepared from laboratory-separated pericarp gave 48% xylose, 35% arabinose, and 7% galactose. The remaining 10% of unidentified material presumably contained the uronic acid. The ratio of arabinose to xylose that was found is comparable to that reported for the hemicelluloses of flours (14) and of endosperm cell walls of wheats (21). The arabinose:xylose ratio of hemicelluloses in the kernels of cereal grains is apparently considerably higher than that of the hemicelluloses of cereal straws (1) and of corncobs (5). It has been reported that the higher the arabinose:xylose ratio of a hemicellulose, the greater is the solubility of the hemicellulose in water (14, 15). The high solubility of the hemicelluloses from corn hull may therefore be attributable to their high arabinose content.

The equivalent weight of the hemicelluloses prepared from laboratory-separated pericarp was in the range of 2,175 to 2,483; the anhydrouronic acid content varied from 7.3 to 8.1%. The equivalent weight of hemicelluloses prepared from several commercial samples of hull was in the range of 1,753 to 2,716; the anhydrouronic acid content ranged from 6.5 to 12.2%. The hemicelluloses had apparently already undergone deesterification through the action of the hot alkali used to effect their removal from the fibrous tissues; little or no evidence of the presence of esters in the final product was found.

An actual yield of 48% furfural was obtained from a portion of the hemicellulose sample, from pericarp, which was analyzed by paper chromatography. A furfural content of about 52% was calculated from the sum of the xylose and arabinose contents (by chromatography) and the uronic acid content (by titration). In making the calculations,

the conventional value of 40% of theoretical for the conversion to furfural of uronic acid was employed. The calculated yield of 52% furfural would be equivalent to a pentosan content of about 82% expressed as anhydroxylan (factor, 1.58). This is within the range of pentosan content found by analysis of the various raw materials (Table IV).

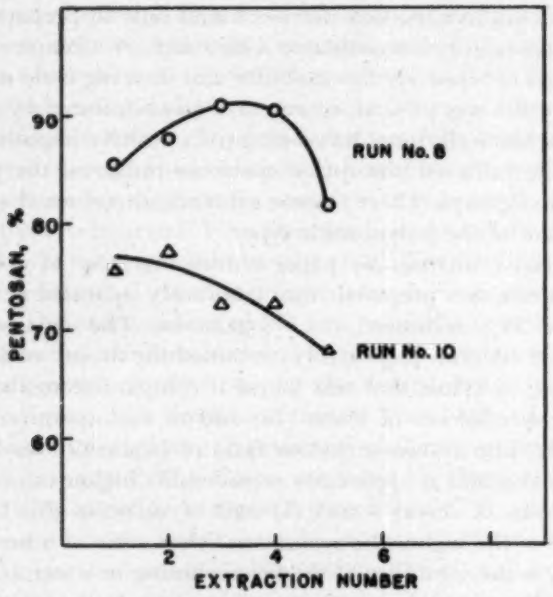

Fig. 2. Pentosan content of successive hemicellulose extracts (ash- and moisture-free basis).

The heterogeneity of the extracted hemicelluloses is emphasized by the variation in pentosan content of the material obtained in successive extracts during a run. When fiber was treated with sodium carbonate followed by water extraction, the plot of pentosan content of successive hemicellulose fractions *vs.* extraction number typically reached a maximum at the third extraction (Fig. 2). When sodium hydroxide was used in place of sodium carbonate, the pentosan content tended to decrease in successive extractions (Fig. 2). Other workers obtained similar results in studying hemicelluloses from wheat straw; it was shown that a fractionation occurred when the original holocellulose was treated successively with alkali solutions of increasing strength (6, 9).

Solution Viscosity of the Hemicelluloses. The viscosity of aqueous solutions of the hemicelluloses prepared from corn hull lay in the

same general range regardless of the raw material used and of the method of extraction employed. Boiling the solution of the isolated hemicelluloses at pH 5.3 resulted in practically no reduction in their viscosity, but autoclaving at the same pH caused a slight reduction in viscosity (Fig. 3), probably as the result of hydrolysis. However,

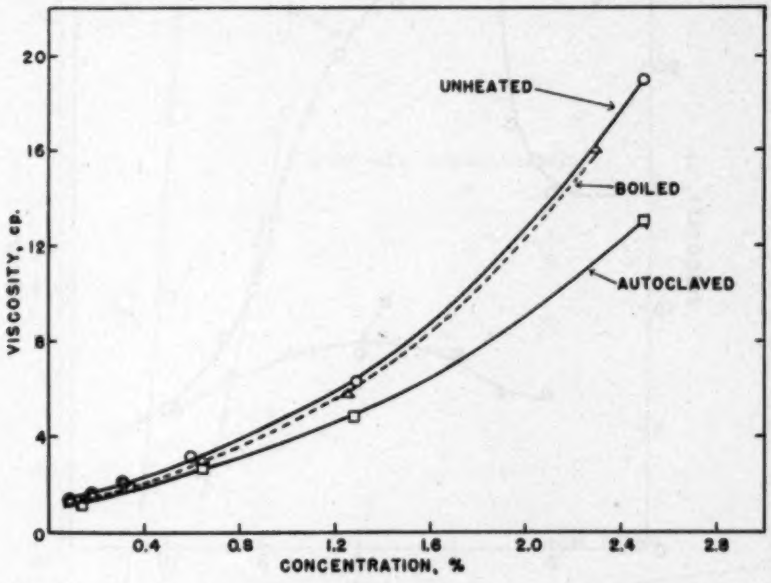

Fig. 3. Effect of boiling (15 min.) and of autoclaving (30 min. at 15 psi, 121°C.) on viscosity of corn-hull hemicellulose solutions.

salt content, pH, and concentration (0.5 to 5%) of the hemicellulose solutions had a marked effect on their viscosity. At constant pH, the viscosity of the aqueous solutions at first decreased rapidly and then approached a constant value as the salt content of the system was increased. The viscosity of the free acid in salt-free, dilute hydrochloric acid solutions was relatively low and rose sharply to a maximum around pH 3.5 (Fig. 4, Curve A). The viscosity decreased as the pH was further increased and the sodium salt of the hemicelluloses was formed. When an excess of sodium chloride was present in the system, the viscosity was lowered over the entire pH range (Fig. 4, Curve B). As in the case of the salt-free system, the viscosity-pH curve passed through a maximum.

The viscosity of aqueous solutions of the hemicelluloses increased rapidly with concentration. A typical viscosity-concentration curve of corn-hull hemicelluloses is compared with those of karaya gum and

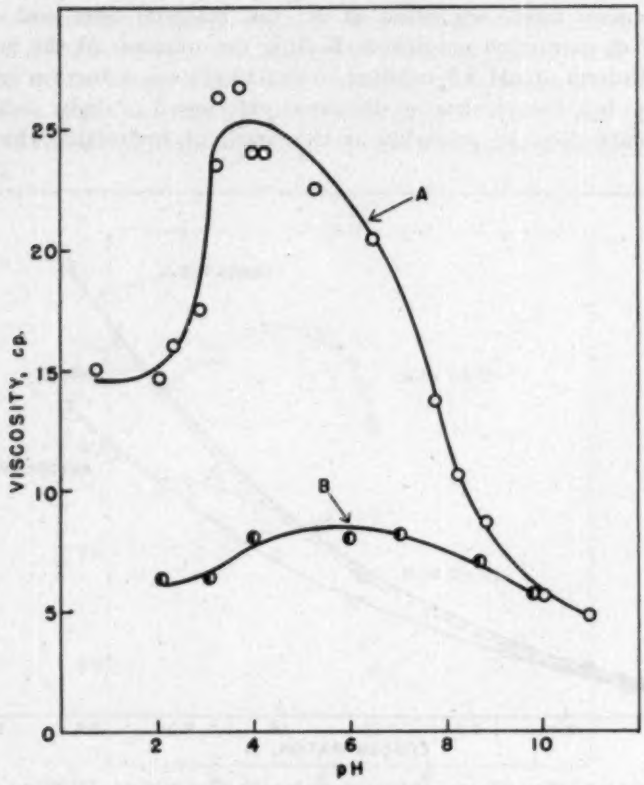

Fig. 4. Effect of pH on viscosity of hemicellulose solutions of different initial ash content. A. 1.7% hemicellulose solution; initial ash content of sample, 0.18%. B. 1.8% hemicellulose solution; initial ash content of sample, 3.2%.

gum arabic in Fig. 5. At all concentrations studied, the hemicellulose solutions were intermediate in viscosity between the karaya-gum and gum-arabic solutions. Gum-tragacanth solutions were found to be of about the same viscosity as those of karaya gum, when the solutions of both gums were prepared in a high-speed blendor at room temperature. Dispersions of locust-bean gum and guar flour, similarly prepared, had higher viscosities than were found for any of the other materials studied.

Ease of Dispersion in Water. The corn-hull hemicelluloses dispersed in water at room temperature to give clear solutions with little or no evidence of the presence of aggregates. In contrast, karaya, tragacanth, and locust-bean gums and guar flour dispersed with difficulty, and even after autoclaving at 15 psi for 20 minutes their solutions contained swollen masses, evident upon microscopic examination. Gum arabic

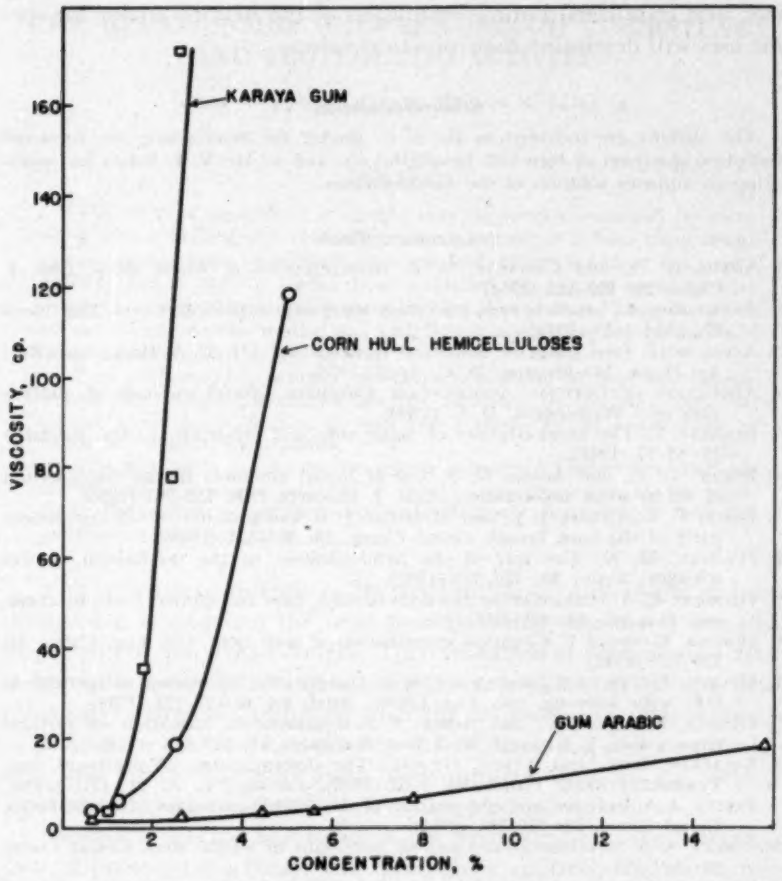

Fig. 5. Comparison of solution viscosity of corn-hull hemicelluloses with that of gum arabic (U.S.P. Select), and karaya gum (white grade, super initial powdered).

dispersed easily and, of the commercial gums studied, was most like the corn-hull hemicelluloses in this respect.

Possibilities for Utilization. There appears to be a promising possibility of converting into a valuable specialty product a portion of the corn hull which is now added to high-protein feed. A third, or more, of the hull can be separated as the hemicelluloses, which have physical properties within the range of those of the commercial vegetable gums. The corn-hull hemicelluloses surpass many of the commercial vegetable gums in ease and completeness of dispersion in water, and the aqueous hemicellulose solutions are stable over a wide pH range. Corn-hull hemicelluloses might well prove useful as thickeners, adhe-

sives, and stabilizers. Future evaluation of the hemicelluloses for specific uses will determine their practical value.

Acknowledgments

The authors are indebted to Dr. S. C. Burket for determining the infra-red absorption spectrum of corn-hull hemicelluloses, and to Mr. V. E. Sohns for spray-drying an aqueous solution of the hemicelluloses.

Literature Cited

1. Adams, G. A., and Castagne, A. E. Hemicelluloses of wheat straw. Can. J. Chem. **29:** 109–122 (1951).
2. Anonymous. By-products recovered from wood chip explosion. Chem. Eng. News **27:** 1534–1535 (1949).
3. Anonymous. Feed statistics. Statistical Bulletin No. 111. U. S. Dept. Agr., Bur. Agr. Econ. Washington, D. C. April, 1952.
4. Association of Official Agricultural Chemists. Official methods of analysis (7th ed.). Washington, D. C. (1950).
5. Bennett, E. The hemicelluloses of maize cobs and rye straw. J. Agr. Research **75:** 43–47 (1947).
6. Bishop, C. T., and Adams, G. A. Use of liquid ammonia in the fractionation of wheat straw holocellulose. Can. J. Research **28B:** 753–761 (1950).
7. Earle, F. R., Curtis, J. J., and Hubbard, J. E. Composition of the component parts of the corn kernel. Cereal Chem. **23:** 504–511 (1946).
8. Fineman, M. N. The role of the hemicelluloses in the mechanism of wet strength. Tappi **35:** 320–324 (1952).
9. Flanders, C. A. Hemicellulose fractions of some hays and straws. Arch. Biochem. and Biophys. **36:** 425–429 (1952).
10. Hooper, Florence E. Chemical examination of corn bran. Ind. Eng. Chem. **34:** 728–729 (1942).
11. Hughes, Elizabeth E., and Acree, S. F. Quantitative estimation of furfural at 0°C. with bromine. Ind. Eng. Chem., Anal. Ed. **6:** 123–124 (1934).
12. Hughes, Elizabeth E., and Acree, S. F. Quantitative formation of furfural from xylose. J. Research Natl. Bur. Standards **21:** 327–336 (1938).
13. Kullgren, Carl, and Tyden, Hilding. The determination of pentosans. Ing. Vetenskaps Akad. Handl. **94:** 3–62 (1929). *Through* C. A. **24:** 1316 (1930).
14. Perlin, A. S. Isolation and composition of the soluble pentosans of wheat flours. Cereal Chem. **28:** 370–381 (1951).
15. Perlin, A. S. Structure of the soluble pentosans of wheat flour. Cereal Chem. **28:** 382–393 (1951).
16. Porst, C. E. G. Occurrence of pentosans and hydrolyzed pentosans in corn and corn products. Orig. Com. 8th Intern. Congr. Appl. Chem. **13:** 205–212 (1912).
17. Schulze, E. Zur Chemie der pflanzlichen Zellmembranen. III. Hoppe-Seyler's Z. physiol. Chem. **19:** 38–69 (1894).
18. Thompson, J. O., and Wise, L. E. Some molecular properties of the hemicelluloses of big tooth aspen. Tappi **35:** 331–336 (1952).
19. Winkler, L. W. Beitrag zur titrimetrischen Bestimmung des Ammoniaks. Z. angew. Chem. **26:** 231–232 (1913).
20. Wolf, M. J., Buzan, C. L., MacMasters, M. M., and Rist, C. E. Structure of the mature corn kernel. II. Microscopic structure of pericarp, seed coat, and hilar layer of dent corn. Cereal Chem. **29:** 334–348 (1952).
21. Wolf, M. J., Seckinger, H. L., Rosewall, E. C., MacMasters, M. M., and Rist, C. E. Studies of water-insoluble hemicelluloses of the endosperm cell walls in relation to milling quality of seven Pacific Northwest wheat varieties. Cereal Chem. **29:** 399–406 (1952).

THE RELATIONSHIP BETWEEN DOUGH CONSISTENCY AND PROTEOLYTIC ACTIVITY[1]

JOHN A. JOHNSON[2] AND BYRON S. MILLER

ABSTRACT

The effect of proteolysis in doughs may be directly evaluated by measuring with a Farinograph the changes in consistency of a flour-water dough containing an excess of purified alpha-amylase during a 4-hour rest period at 30°C. and at pH 4.7. Under these conditions, the change in dough consistency was proportional to the concentration of proteinase. The pH optima for the enzyme systems studied was 4.12. Dough consistency change increased linearly between 25° and 40°C.

Data confirmed the presence of a papain inhibitor in flour and demonstrated that fungal proteinases are not inhibited by oxidants, but that malted wheat flour contains at least two proteolytic components, one of which is inhibited by oxidants.

The method is limited to the testing of enzyme preparations that are not affected by a papain inhibitor in flour. It also is not suitable for measuring the activity of preparations, such as patent flour, which have low proteinase concentration.

The widespread interest in the application of proteinases in bread doughs has emphasized the need for evaluating objectively the effect of proteolytic activity in doughs. The estimation of proteolytic activity is normally based either on viscometric procedures or on the determination of products resulting from the proteolysis of such substrates as gelatin, casein, and hemoglobin. All of these are of animal origin and may differ markedly from flour protein in both the relative amounts of amino acid constituents and physical structure.

Landis (4) was the first to adapt the Farinograph to the determination of proteolytic activity. One proteolytic enzyme unit was defined as "that amount of enzyme which, when present in the standard dough, will produce an initial rate of decrease in development energy of 1000° B-minutes per hour." The method specifies the addition of sodium chloride to give a final concentration of 1.0% salt based on flour. Sodium chloride, however, has been demonstrated to be an inhibitor of proteolytic activity (6). In the method used by Landis, the entire contact time of the flour and enzyme was 37 minutes, exclu-

[1] Manuscript received December 1, 1952. Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station. Contribution No. 220, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station.

[2] The results reported will be developed as part of a thesis to be submitted by John A. Johnson in partial fulfillment of the requirements for the Ph.D. Degree at the University of Minnesota, St. Paul 1, Minnesota.

sive of the time required to mix the dough to maximum consistency.

Johnson and Miller (3) employed a Farinograph technic to evaluate the effect of enzyme components on sponge dough consistency. After fermentation for 4 hours, a portion (530 g.) of flour-water-enzyme dough was placed in the large Farinograph bowl and the consistency followed during a 15-minute mixing period. The change in dough consistency due to enzyme action after 15 minutes of mixing was taken as a measure of enzymatic changes in the dough. These studies revealed that both alpha-amylase and proteinase were responsible for the decrease in dough consistency. The effect of alpha-amylase was limited, however, by the amount of starch which was susceptible to attack. A decrease in dough consistency of 12 to 20% could be attributed to the action of alpha-amylase alone. The change in dough mobility was found to be correlated directly with the concentration of proteinase.

These studies (3) suggested that changes in dough consistency could be used as a measure of the proteolytic activity of various enzyme preparations. The effect of alpha-amylase on such changes may be made a constant by use of a standard flour and by addition of an excess of alpha-amylase, freed of contaminating proteinase. By this means any changes in dough consistency might be directly related to the concentration of proteinase of the enzyme supplement acting under standardized conditions of pH, time, and temperature.

Since the use of auxiliary substrate in methods measuring various end-products of proteolysis may be related only remotely to the effect which proteinases have in bread dough, the present investigation was made to develop a means of measuring directly the effect of proteinases using bread dough sponges. Since pH, time, temperature, and possibly oxidants may affect the rate of proteolysis, this investigation was designed to study these factors.

Proteinases decrease the consistency of the sponge dough during fermentation, which may be followed conveniently with a Farinograph. The problem was then to study the factors that might be related to the action of the proteinase on sponge consistency.

Materials. An unmalted, commercially milled, hard red winter wheat patent flour (protein 11.8%, ash 0.41%) was used as a substrate.

Five proteinase enzyme preparations included commercial crude papain,[3] malted wheat flour,[4] commercial crude pepsin,[3] and the fungal enzymes, Rhozyme-S[5] and Rhozyme P-11.[5] Rhozyme-S was produced

[3] Merck & Co., Rahway, New Jersey.
[4] Kansas Milling Co., Wichita, Kansas.
[5] Rohm & Haas Co., Philadelphia, Pa.

using *Aspergillus oryzae* and had a high ratio of amylase to proteinase compared to that for Rhozyme P-11. The enzymes were extracted with water just prior to use. While the enzymes may be added to the flour in dry form, the use of extracts insured more uniform incorporation in the dough. An alpha-amylase, freed of proteinase, was prepared from Rhozyme-S by the method of Miller and Johnson (7). This alpha-amylase was added to each sponge dough in order that an excess would be present, thus neutralizing the effect of the added alpha-amylase from the various test supplements.

The Farinograph with the large bowl was used for all consistency measurements. Preliminary studies suggested that the peak consistency was not so consistent a measurement of the effect of the proteinase as was the consistency after 15 or more minutes of mixing. Routinely, the consistency of sponges was measured after 15 minutes of mixing.

Sponge doughs were routinely made by mixing 490 g. of flour in a Hobart A-200 for 2 minutes, with enough water to provide a dough having a predetermined maximum consistency of 500 BU. Since oxidants are known to affect dough consistency they were omitted except where their effect was being studied. Likewise, sodium chloride was omitted since it has been shown to have an inhibitory effect on the proteinases of cereal and fungal preparations. Sodium chloride also affects the colloidal properties of dough.

The sponge dough was placed in a fermentation cabinet or in stone crocks placed in a water bath at the desired temperature for 4 hours, an amount of time approximating that of commercial sponge-dough baking procedures.

Effect of pH on Changes in Dough Consistency. The effect of pH on proteolytic activity in sponge doughs was studied using fungal proteinase. The pH of the dough was adjusted by the addition of various quantities of 1.0 *N* acetic acid. This acid was mixed with three-fourths of the required amount of water and added to the flour. After 0.5 minute of mixing at low speed the enzyme was added and mixing continued for 1.5 minutes at second speed. The pH of the doughs was measured by placing the glass electrode directly in the dough. The temperature of the water was adjusted so that the doughs came out of the mixer at 30°C.

The effect of pH on proteolytic activity in sponge doughs is shown in Fig. 1. The optimum activity for this enzyme source was at pH 4.12, which corresponds well with the optimum obtained for hemoglobin (5) or gluten substrate[6]. Since these latter methods employ pH 4.7 and since it seemed desirable to compare the results of changes on

[6] Unpublished work from this laboratory.

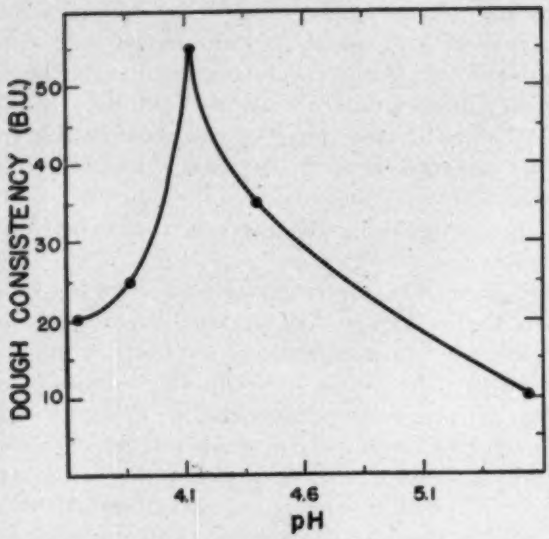

Fig. 1. Effect of pH on the activity of Rhozyme P-11 freed of alpha-amylase in sponge dough (14.7 mg./490 g. of flour).

sponge doughs with enzyme activity measured by other methods in subsequent work, pH 4.7 was established as being satisfactory, although greater activity resulted with lower pH values. While the pH of a normal sponge dough (5.2) could have been selected, the sensitivity of the method at this pH was too low to be practical.

Effect of Temperature on Proteolytic Activity in the Sponge. The effect of temperature on proteolytic activity in the sponge was investi-

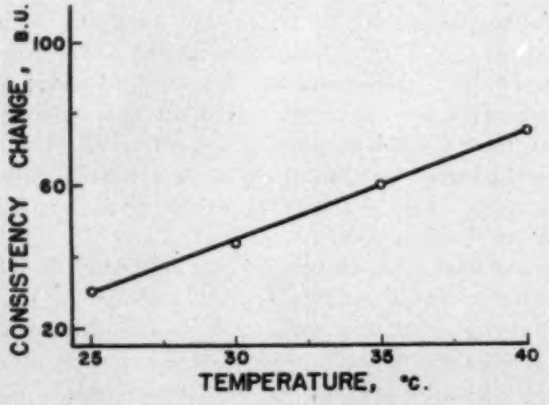

Fig. 2. Effect of temperature on the action of Rhozyme P-11 freed of alpha-amylase (20 mg./490 g. of flour) in dough.

gated by allowing the doughs to react at the different temperatures shown in Fig. 2. These data were obtained at pH 4.7, using Rhozyme P-11 proteinase that had been freed of alpha-amylase. The consistency of the sponges decreased directly as the temperature of reaction was raised between the limits of 25° and 40°C. Experimental difficulties precluded the possibility of using temperatures above 40°C., but the optimum activity is probably considerably higher than 40°C. Similar data have been reported by Miller (5) for the Ayre-Anderson method employing bacto-hemoglobin substrate.

Effect of Proteinase Concentration on Changes of Sponge Dough Consistency. To be of most value, a method of measuring proteolytic activity should reflect the effect of enzyme concentration directly or be capable of transformation to provide a linear relationship. The effect of Rhozyme P-11 proteinase concentrations freed of alpha-amylase on the changes in sponge dough consistency resulting from digestion for 4 hours at pH 4.7 and 30°C. is shown in Fig. 3. The

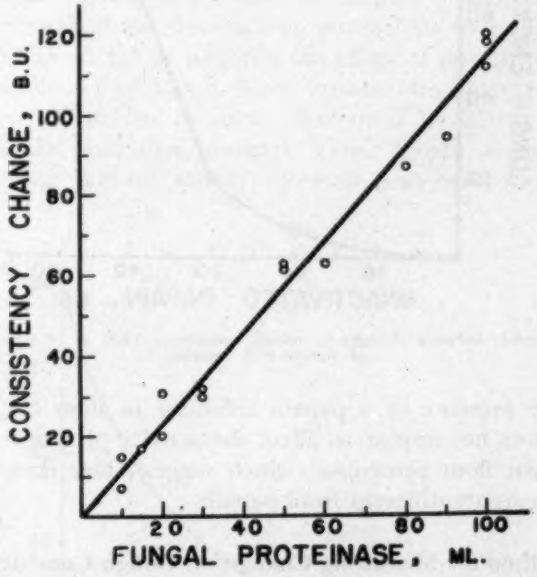

Fig. 3. The relationship between changes in sponge dough consistency (490 g. of flour) and quantity (10 ml. = 14.7 mg.) of Rhozyme P-11 freed of alpha-amylase.

correlation coefficient of 0.99 (13 d.f.) and a standard deviation from regression of 5.25 BU were calculated for these data. Similar linear relationships were found for Rhozyme-S, pepsin, and malted wheat flour proteinase freed from alpha-amylase.

In experiments with commercial papain, the enzyme was not activated with the customary activators because these have an effect on the colloidal dough properties which is independent of the extent of proteolysis. The relationship between concentration of commercial papain and changes in dough consistency were not linear until approximately 30 mg. of the preparation per 490 g. of flour had been added (Fig. 4). This lack of linearity is not surprising, however, in

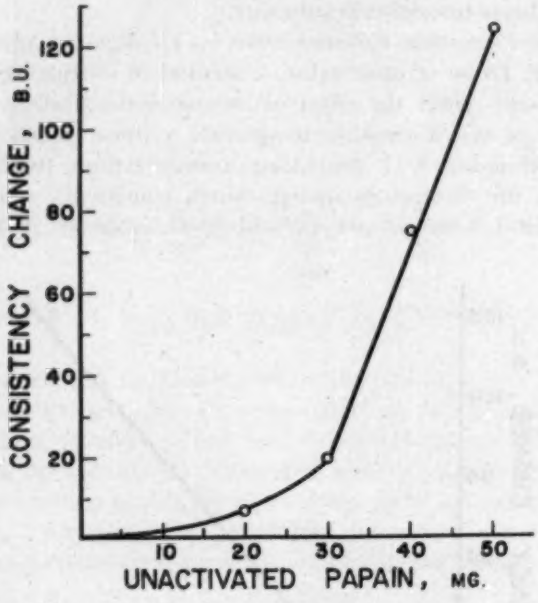

Fig. 4. Relationship between changes in dough consistency (490 g. of flour) and quantity of unactivated papain.

view of the presence of a papain inhibitor in flour (2). This same inhibitor does not appear to affect the activity of fungal, pepsin, or malted wheat flour proteinase, which suggests that these latter proteinase systems are different from papain.

Method for Measuring Changes in Dough Consistency Due to Proteolytic Activity

As a result of the studies described above, the following procedure was developed to measure proteolytic effects in sponge doughs.

Sponge doughs consisting of 490 g. of flour, three-fourths of the optimum amount of water predetermined by the Farinograph curve (500 BU consistency), and 20 cc. of 1.0 *N* acetic acid are mixed for 0.5

minute at low speed in a Hobart A-200 Mixer. The remainder of the water, 300 units of alpha-amylase freed of proteinase, and the desired amount of supplement (in all doughs except the blank) are added and the dough is mixed for 1.5 minutes at second speed. Duplicate doughs are allowed to rest for 4 hours in covered containers at 30°C. and 85% relative humidity. A piece of each dough (530 g.) is weighed into the large bowl of the Farinograph and consistency followed during 15 minutes of mixing. The consistency of the dough containing no proteinase should range between 300 and 350 BU. The difference in consistency after 15 minutes of mixing of a dough containing no added proteinase and one containing added proteinase supplement is taken as a measure of proteolytic activity. The amount of enzyme employed should not be greater than the amount required to lower the consistency more than 100 units.

The Effect of Oxidants on Proteolytic Activity in Sponge Doughs

Although the Farinograph consistency technic is not recommended as a routine method for determining proteolysis in doughs, it has proved useful as an aid in studying the effect of certain ions on the action of proteinases in dough. Since various ions alter the colloidal dough properties, studies of their effect must be determined independently of the proteinase enzymes. Thus, doughs containing only potassium iodate but no added proteinase enzyme will show ionic

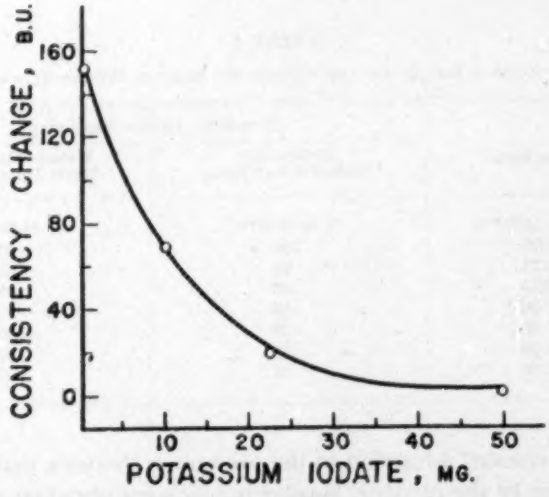

Fig. 5. Effect of potassium iodate (mg./500 g. flour) on papainase activity (120 mg. papain/490 g. of flour).

effects as evidenced by a change in dough consistency, particularly at high concentrations (25 mg. % of potassium iodate or higher). Doughs containing equivalent amounts of the oxidant were used as blank determinations in evaluating the consistency of doughs containing added proteinase and oxidant. Figure 5 shows that potassium iodate has a marked effect on the action of papain in dough. Similar inhibition was demonstrated for the action of papain on hemoglobin or gluten substrates using the Ayre-Anderson procedure. In contrast with these results for papain, no inhibitory effect of potassium iodate could be demonstrated for fungal (Rhozyme P-11 or Rhozyme-S) proteinase as measured by the Farinograph technic, or by the modified Ayre-Anderson technic (5), employing either hemoglobin or gluten substrates. This was true even though the amount of potassium iodate used was 100 times that employed in the Farinograph technic based upon the amount of protein involved.

Studies involving the effect of potassium iodate on the action of malted wheat flour proteinase produced interesting results. Potassium iodate inhibited a small fraction of the activity of commercial malted wheat flour, but no inhibitory effect could be observed if the enzyme extracts were treated to remove the alpha-amylase, according to the differential inactivation technic of Miller and Johnson (7). These observations were confirmed with the Ayre-Anderson (5) technic using gluten as the substrate. An example of this inhibition is shown in Table I. It would appear likely that the purification procedure (7)

TABLE I

EFFECT OF POTASSIUM IODATE ON THE ACTION OF MALTED WHEAT FLOUR ON GLUTEN

Potassium Iodate	Proteolytic Activity Remaining	
	Commercial Malted Wheat Flour	Malted Wheat Flour Minus Alpha-Amylase
mg/20 g. gluten	*% of control*	*% of control*
0.00	100	100
0.25	97	95
0.50	63	97
1.00	53	95
5.00	56	100
50.00	53	100
100.00	56	100

may have removed a fraction of the proteinase complex that is subject to the effects of the oxidant. Similar results were obtained with potassium bromate. These results suggest that there are at least two pro-

teinases in commercial malted wheat flour, one of which is inhibited by oxidants.

Acknowledgment

Grateful acknowledgment is made to Kansas Flour Mills, Kansas City, Missouri, for the unmalted flour, and to Rohm and Haas Company, Philadelphia, for the Rhozyme-S and Rhozyme P-11 used in this investigation.

Literature Cited

1. Bowlby, C. M., Tucker, H., Miller, B. S., and Johnson, J. A. A comparison of methods for determining proteolytic activity. Cereal Chem. **30:** 480–491 (1953).
2. Hites, B. D., Sandstedt, R. M., and Schaumburg, Lorene. Study of proteolytic activity in wheat flour doughs and suspensions. II. A papain inhibitor in flour. Cereal Chem. **28:** 1–18 (1951).
3. Johnson, J. A., and Miller, B. S. Studies on the role of alpha-amylase and proteinase in breadmaking. Cereal Chem. **26:** 371–383 (1949).
4. Landis, Q. An index of proteolytic activity by the use of the Farinograph. Cereal Chem. **12:** 25–33 (1935).
5. Miller, B. S. A critical study of the modified Ayre-Anderson method for the determination of proteolytic activity. J. Assoc. Offic. Agr. Chemists **30:** 659–669 (1947).
6. Miller, B. S., and Johnson, J. A. High levels of alpha-amylase in baking. II. Proteolysis in straight and sponge doughs. Cereal Chem. **25:** 178–190 (1948).
7. Miller, B. S., and Johnson, J. A. Differential stability of alpha-amylase and proteinase. Cereal Chem. **26:** 359–371 (1949).
8. Miller, B. S., and Johnson, J. A. A simple linear relationship and definition of a unit for proteinase activity. Arch. Biochem. and Biophys. **32:** 200–206 (1951).

A COMPARISON OF METHODS FOR DETERMINING PROTEOLYTIC ACTIVITY[1]

CAROL BOWLBY[2], HENRY TUCKER, BYRON S. MILLER, AND JOHN A. JOHNSON

ABSTRACT

Several proteolytic methods and substrates were investigated to determine the ones which are most convenient and reliable as a means of evaluating protease activity in doughs. The following proteolytic methods were investigated: the Ayre-Anderson procedure employing either bacto-hemoglobin or gluten substrate, and Kjeldahl as well as spectrophotometric determinations of soluble nitrogen; the bromsulphalein method; the Farinograph consistency technic; a formol titration procedure; and the milk-clotting technic. The enzyme preparations investigated included malted wheat flour, papain, trypsin, a bacterial and three fungal concentrates.

The Farinograph technic was employed as a reference method with which other procedures were compared. Indirect comparisons of the results obtained by the Ayre-Anderson digestion of hemoglobin using either Kjeldahl or spectrophotometric determinations of soluble nitrogen, the bromsulphalein method, and the formol titration procedure indicate that these methods give results essentially similar to those obtained with the Farinograph. The failure of the technics involving gluten digestion and the clotting of milk to give results similar to those obtained with the other methods emphasizes the importance of substrate, hydrogen-ion concentrations, and type of activity measured in proteolytic determinations.

The enzyme preparations may be classified into two groups on the basis of their action on different substrates: 1) the animal and bacterial preparations; 2) fungal preparations and malted wheat flour.

Numerous methods have been used for the measurement of proteolytic activity. However, due to the host of possible substrates and specific enzymes employed, considerable confusion exists as to which method (or methods) provides the best approximation of true activity as related to dough modification during fermentation. Among the substrates used for proteolytic determinations are casein, gelatin, gluten, hemoglobin, and various protein-dye complexes. Methods used to follow changes in the substrate include such determinations as soluble nitrogen, clotting time, and light absorption.

Several proteolytic methods were studied comparatively by Hildebrand (7,8). One of these studies (8) indicated that the gelation-rate procedure of Landis and Frey (12), the viscometric method of Koch,

[1] Manuscript received December 1, 1952. Cooperative investigations between the Department of Flour and Feed Milling Industries, the Statistical Laboratory, Kansas Agricultural Experiment Station, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture. Contribution No. 226, Department of Flour and Feed Milling Industries, and No. 9, the Statistical Laboratory, Kansas Agricultural Experiment Station.

[2] Present address: Northern Regional Research Laboratory, Peoria, Illinois.

Nelson, and Ehrnst (11), and the Ayre-Anderson method (3) gave essentially similar results for the proteolytic activity of cereal products. The results obtained with these methods were not well correlated with those obtained by Sorenson's formol titration procedure using flour or gelatin as a substrate (7). Hildebrand suggested that physicochemical rather than chemical methods may give a better measurement of dough modification by proteolytic action.

Among the methods on which little or no compartive work has been done are many colorimetric, spectrophotometric, and physicochemical methods. The colorimetric procedure proposed by Greif (6) using bromsulphalein for the quantitative precipitation of undigested protein appeared to have the advantages of convenience and adaptability for routine analysis. Abbott, Miller, and Johnson (1) described a spectrophotometric modification of the Ayre-Anderson procedure which was highly correlated with the determination of soluble nitrogen by the usual Kjeldahl procedure. Physico-chemical methods of proteolytic analysis include the milk-clotting technic (4), and the Farinograph technic (13).

Since interest in the use of proteinase in breadmaking is assuming more and more importance, this work was undertaken to determine which methods are the most convenient and reliable as a means of evaluating the action of proteinase in doughs.

Materials and Methods

The proteolytic preparations included a malted wheat flour (MWF), papain, trypsin, a bacterial proteinase (Protease-15)[3], and three fungal concentrates, Rhozyme P-11, Rhozyme-S, and Protease-26[3]. Two of the fungal preparations, Rhozyme-S and Protease-26, were prepared from *Aspergillus oryzae* by different procedures.

The Farinograph technic was used essentially as described by Miller and Johnson (13). This procedure is not applicable to the analysis of papain activity due to the presence of a papain inhibitor in flour (9).

The modified Ayre-Anderson method and the spectrophotometric modification employing either hemoglobin or gluten as substrate were employed as outlined by Abbott, Miller, and Johnson (1). It was necessary to increase the gluten concentration to 6% (3.0 g.) to avoid the condition of limiting substrate for the levels of enzyme employed.

A formol titration procedure based on that outlined by Koch (10) was used. The substrate employed was a 10% solution of hemoglobin in acetate buffer (pH 4.7). Equal volumes of substrate and enzyme solution were mixed and digested for 1 hour at 30°C. Before and after

[3] P-15, P-11, Rho-S, and P-26, obtained from Rohm and Haas Co., Philadelphia, Pa.

digestion, 20-ml. aliquots of the mixture were added to 15 ml. of neutralized formalin solution (pH 8.5) and titrated to pH 8.5 with standard sodium hydroxide employing Beckman glass electrode equipment. Proteolytic activity was expressed as the increase in titration value of 0.05 *N* sodium hydroxide. Several of the enzymes were omitted from these determinations because of inactivity or interfering material in the preparations themselves.

The bromsulphalein procedure reported by Greif (6) was modified. A 10% solution of hemoglobin in acetate buffer (pH 4.7) was used as the substrate. Preliminary experiments indicated that maximum precipitation of the substrate occurred when a 0.25% solution of bromsulphalein (disodium phenoltetrabromphthalein disulfonate)[4] in citrate-phosphate buffer of pH 3.0 was employed. The reactions were carried out at 30°C. for 1 hour. The decrease in optical density of the reaction mixtures during digestion was taken as a measure of proteolytic activity.

The milk-clotting technic employed was essentially the same as that described by Balls and Hoover (4). The malted wheat flour was not analyzed by this method owing to the relative inactivity of the preparation.

Five or six levels of each preparation were analyzed in duplicate on different days by each method, except where noted.

Results

Enzyme concentrations were plotted against corresponding activity values for each of the methods and the graphs examined to determine the basic relationships. For the ranges of enzyme concentration used, the functions could be described as linear, except for those obtained with the bromsulphalein method, which were linear when the optical density measurements were transformed (raised to the 3/2 power) and plotted against enzyme concentration. Examples of the type of relationsbips observed are shown in Figs. 1 and 2.

The data obtained by the bromsulphalein method were markedly curvilinear for all the systems investigated. By use of a square transformation, the papain data followed a linear trend (Fig. 3) while the 3/2 power was found to be equally suitable for Rhozyme P-11, Rhozyme-S, Protease-26, and malted wheat flour (Fig. 1). No suitable transformation was found for the bacterial proteinase, Protease-15.

The regression coefficients between enzyme concentration and activity were calculated for each of the enzymes and methods. The regression coefficients between these two variables investigated for each

[4] Hynson, Wescott, & Dunning, Inc., Charles & Chase Streets, Baltimore, Md.

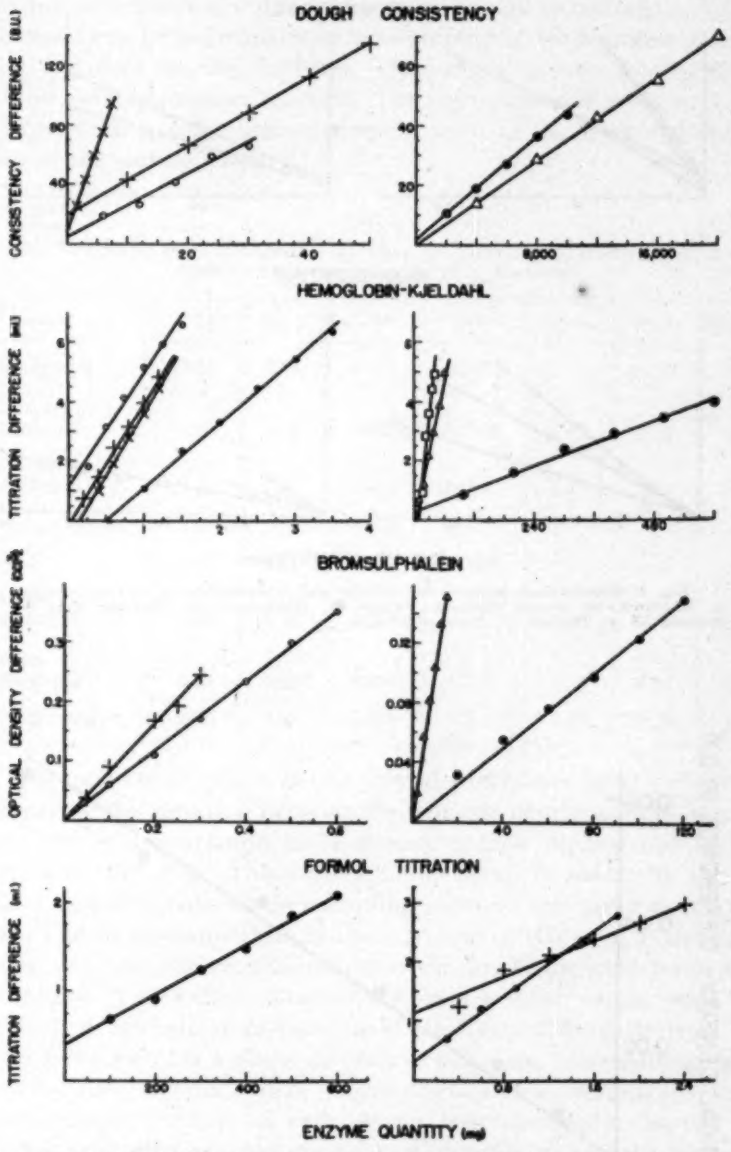

Fig. 1. Relationships between the activity and concentration of several enzymes as measured by several methods. Papain ●, Rhozyme-S ○, Rhozyme P-11 +, Protease-15 ×, Trypsin □, Protease-26 △, and malted wheat flour ⊙.

enzyme on two days were not significantly different. In almost all instances, the intercept (the activity for the particular method when the

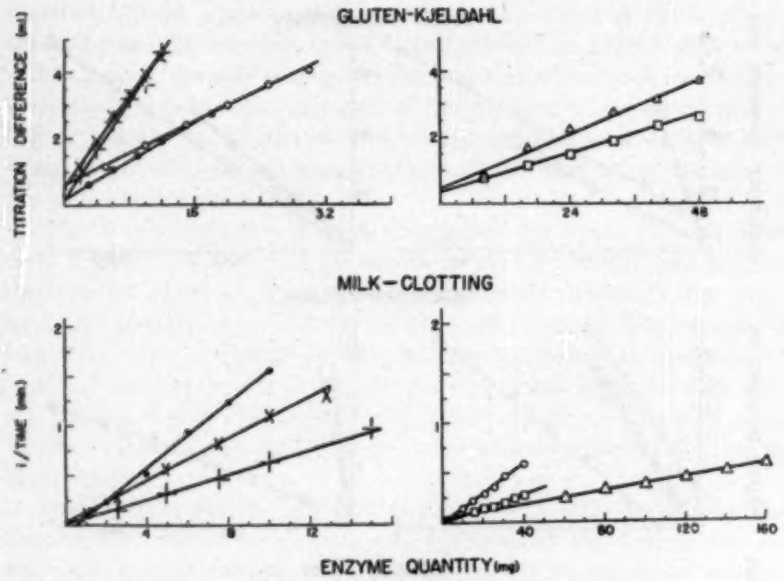

Fig. 2. Relationships between the activity and concentration of several enzymes as measured by several methods. Papain ●, Rhozyme-S O, Rhozyme P-11 +, Protease-15 ×, Trypsin □, Protease-26 △.

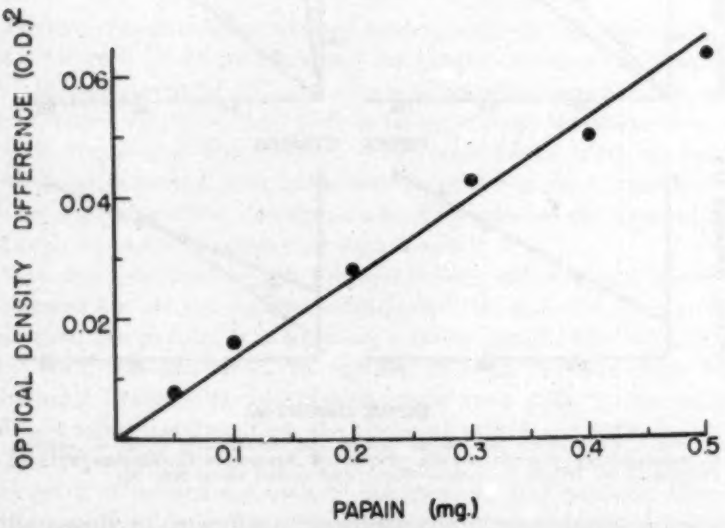

Fig. 3. Relationship between the activity and concentration of papain as measured by the bromsulphalein method.

enzyme concentration was zero) was close enough to the origin for the differences to be regarded as of no significance. Under these assumptions the data for the duplicate levels and days were pooled to establish a single response function. The regression coefficients in Table I express the average activity rates in units of the method for each unit of the enzyme used.

TABLE I

COEFFICIENTS OF REGRESSION BETWEEN ENZYME CONCENTRATION AND ACTIVITY DETERMINED BY SEVERAL METHODS

Method	P-11	Rho-S	P-26	MWF	Papain	P-15	Trypsin
Farinograph	2.29	2.13	0.036	0.0043	...	11.30	0.473
Hemoglobin-Kjeldahl	4.12	3.80	0.086	0.0062	2.09	4.23	0.166
Hemoglobin-Beckman	0.22	0.18	0.004	0.0003	0.10	0.21	0.011
Bromsulphalein	0.82	0.59	0.010	0.0013	...	...	.....
Formol	0.80	1.37	....	0.0029	...	...	....
Gluten-Kjeldahl	3.66	1.18	0.068	.....	1.39	3.33	0.048
Gluten-Beckman	0.18	0.03	0.004	.....	0.03	0.12	0.003
Milk-clotting	0.06	0.02	0.004	.....	0.17	0.10	0.007

The relative precision of the several procedures may be seen by comparing the variation between the replicate determinations at each level and by the variation of the average of these replicates about the regression line (14). The former comparison is made by use of $(C)_e = s_e/\bar{y}$, the ratio of the sampling error to the mean concentration. The latter comparison is made by use of $(C)_r = s_r/\bar{y}$, the ratio of the standard deviation about the trend line to the same mean concentration. The values obtained for each enzyme varied with the method of determination being used, and varied similarly from enzyme to enzyme for a given method. While some more suitable procedures, such as rank orders, might produce more statistically valid results, simple averages for each method were obtained to express the average variability which is encountered in using each of the methods. These data are given in Table II. While all preparations were not used for each method, the relatively high values for $(C)_e$ for the Farinograph and bromsulphalein methods indicate the need for a greater number of replications if the precision of the other methods is

TABLE II

SUMMARY OF STATISTICAL CALCULATIONS FOR THE RELATIVE PRECISION OF THE VARIOUS METHODS

Method	$(C)_e$[1]	$(C)_r$[2]
Farinograph	15.5	7.1
Hemoglobin-Kjeldahl	4.1	6.2
Hemoglobin-Beckman	4.1	7.4
Bromsulphalein	16.0	17.0
Formol	6.0	3.5
Gluten-Kjeldahl	9.3	5.5
Gluten-Beckman	7.0	2.8
Milk-clotting	6.0	4.6

[1] $(C)_e$, coefficient of variation based on the standard deviation of the average activity values for a given enzyme concentration.
[2] $(C)_r$, coefficient of variation based on standard deviation from regression.

to be obtained. With the bromsulphalein method small enzyme quantities are required and small differences in readings of optical density are obtained for different enzyme concentrations. With such small measurements the same physical reading error becomes a larger percentage error; hence the somewhat larger coefficient of variation for this method as compared with the others.

Discussion

In order to make comparisons among methods and enzymes, the number of milligrams of each enzyme which would be needed to produce some average arbitrary level of activity for each method was computed from the regression equations plotted in Figs. 1 and 2. Assuming the intercept is at the origin, the enzyme concentration can be obtained by dividing the arbitrary activity value by the regression coefficient for each particular enzyme and method. For example, using the regression coefficients given in Table I, it would take 21.83 mg. of Rhozyme P-11 or 23.47 mg. of Rhozyme-S to produce an average equivalent effect of 50 Brabender Units with the Farinograph method. These values, presented in Table III, are useful to express the relative activities of the various enzyme preparations measured by a given method.

One direct comparison can be made, however, by correlating the change in soluble nitrogen determined by the Kjeldahl and spectrophotometric procedures. This direct comparison can be made because the values were obtained by using aliquots for analysis from the same digest. The correlation between titration values and optical density were highly significant for each enzyme when either hemoglobin or

TABLE III

MILLIGRAMS OF ENZYME SHOWING EQUIVALENT ACTIVITY FOR A GIVEN METHOD OF ANALYSIS

Method	Activity Base	P-11	Rho-S	P-26	MFW	Papain	P-15	Trypsin
Farinograph	50 B.U.[1]	21.83	23.47	1388.8	11628	...	4.42	105.70
Hemoglobin-Kjeldahl	3 ml.[2]	0.73	0.79	34.9	484	1.44	0.71	18.10
Hemoglobin-Beckman	0.2 O.D.[3]	0.91	1.11	50.0	667	2.00	0.95	18.20
Bromsulphalein	0.2 O.D.[3]	0.24	0.34	20.0	154	...	...	....
Formol	2 ml.[2]	2.50	1.46	...	690	...	...	....
Gluten-Kjeldahl	2 ml.[2]	0.55	1.69	29.4	...	1.44	0.60	41.70
Gluten-Beckman	0.2 O.D.[3]	1.11	6.66	50.0	...	6.66	1.67	66.67
Milk-clotting	0.5 1/T[4]	8.33	25.00	125.0	...	2.94	5.00	71.40

[1] Brabender units (difference).
[2] Titration difference.
[3] Optical density difference.
[4] Reciprocal of clotting time, in minutes.

gluten was used as a substrate. The correlation and regression coefficients are presented in Table IV. These results corroborate the work of Abbott, Miller, and Johnson (1).

Only indirect comparisons of the data involving all methods may be employed, since the quantity of enzyme varied widely between methods and the units of activity have no common basis. The variation in relative activity of the enzymes between methods can be evalu-

TABLE IV

SUMMARY OF THE RELATIONSHIPS BETWEEN OPTICAL DENSITY AND SOLUBLE NITROGEN FOR SEVERAL DIFFERENT ENZYME PREPARATIONS ACTING ON TWO SUBSTRATES

Enzyme Source	Substrate			
	Hemoglobin		Gluten	
	b[1]	r[2]	b[1]	r[2]
Rhozyme P-11	0.0515	0.983	0.0474	0.984
Rhozyme-S	0.0484	0.995	0.0298	0.964
Protease-26	0.0437	0.872	0.0503	0.971
Malted wheat flour	0.0495	0.995	...	...
Papain	0.0479	0.998	0.0203	0.937
Protease-15	0.0468	0.982	0.0366	0.982
Trypsin	0.0587	0.905	0.0701	0.979

[1] Coefficients of regression of optical density on soluble nitrogen.
[2] Coefficients of correlation of optical density on soluble nitrogen. All are significant at the 0.1% level.

ated by comparing the ratios of the enzyme quantities required for equivalent activity when analyzed by the several methods. These data are presented in Table V. For example, the ratio of enzyme quantities

TABLE V

RATIOS OF ENZYME QUANTITIES REQIURED FOR ACTIVITY EQUIVALENT TO THAT OF RHOZYME P-11[1]

Method	P-11	Rho-S	P-26	MWF	Papain	P-15	Trypsin
Farinograph	1	1.08	63.6	533	...	0.20	4.84
Hemoglobin-Kjeldahl	1	1.08	47.8	663	1.97	0.97	24.80
Hemoglobin-Beckman	1	1.22	54.9	733	2.20	1.04	20.00
Bromsulphalein	1	1.42	83.3	642	...	...	....
Formol	1	0.58	...	276	...	...	....
Gluten-Kjeldahl	1	3.07	53.5	...	2.62	1.09	75.80
Gluten-Beckman	1	6.00	4.50	...	6.00	1.51	60.00
Milk-clotting	1	3.00	15.0	...	0.35	0.60	8.57

[1] Based upon data in Table III.

for Rhozyme-S and Rhozyme P-11 using the Farinograph technic is 23.47/21.83 (Table III) or 1.08 (Table V). A similar ratio for the same enzymes analyzed by the hemoglobin-Kjeldahl procedure is 1.08. These activity ratios show the relative activities of the enzymes when analyzed by the various methods.

The data in Figs. 4 and 5 are designed to compare the results obtained by the Farinograph with those obtained by each of the other methods of analysis. Calculated activity values were obtained from the regression equations for each method employing two arbitrary enzyme concentrations. The values have been plotted and lines drawn to indicate the nature of the comparison of results obtained by the various methods. In Fig. 4, generally good agreement is seen for the results obtained by the Farinograph, formol, bromsulphalein, hemoglobin-Kjeldahl, and hemoglobin-Beckman methods for malted wheat flour, Rhozyme-S, Rhozyme P-11, and Protease-26. In several cases, the values plotted in Figs. 4 and 5 were considerably higher than those obtained in actual practice. Although this means that extrapolated values were compared, the agreement is good between the graphic comparison of the calculated data shown in Figs. 4 and 5 and the indirect mathematical comparisons in Table V.

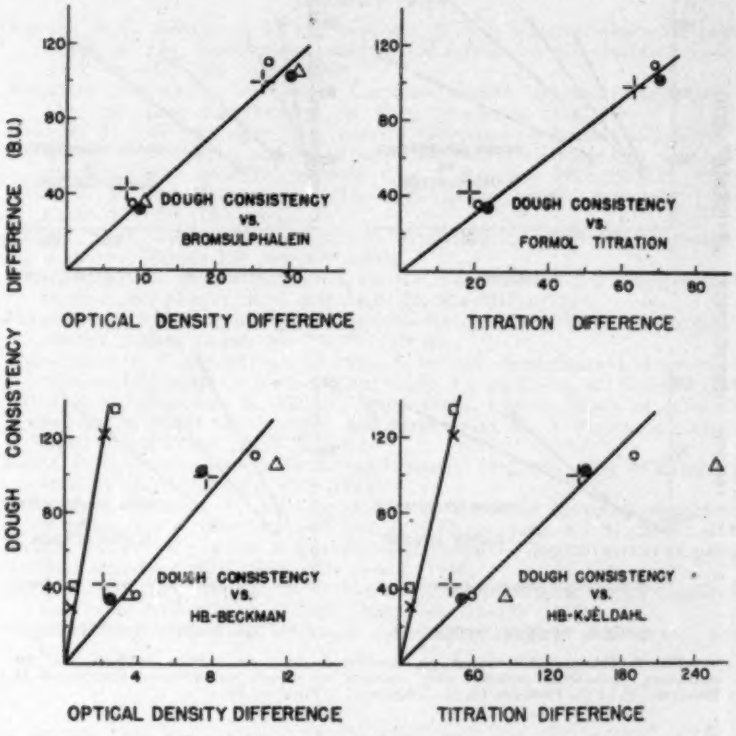

Fig. 4. Comparisons, based on calculated data, of various methods for determining proteolytic activity with changes in dough consistency. Rhozyme-S O, Rhozyme P-11 +, Protease-15 ×, Trypsin □, Protease-26 △, and malted wheat flour ●.

The comparative data (Table V and Figs. 4 and 5) also indicate a similarity of action between trypsin and Protease-15. These results are further evidence of the similarity between bacterial and animal enzymes which was noted previously by Fischer and de Montmollen (5). These enzymes appear to behave similarly when analyzed by the Farinograph, the milk-clotting, and the hemoglobin digestion technics. A comparison of the analyses obtained for all enzymes with methods involving gluten digestion and milk-clotting measurements (Fig. 5 and Table V) shows no consistent trends for these procedures.

No single relationship can be defined relating the results of a particular method to those of the Farinograph for all proteinase preparations. However, when the preparations are divided into two groups, one including the plant and fungal enzymes and the other including animal and bacterial preparations, the results for several

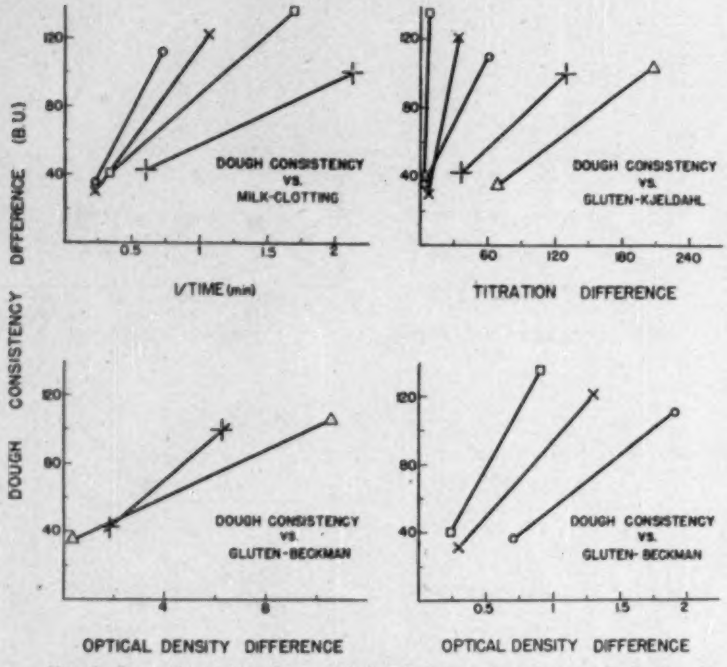

Fig. 5. Comparisons, based on calculated data, of various methods for determining proteolytic activity with changes in dough consistency. Rhozyme-S O, Rhozyme P-11 +, Protease-15 ×, Trypsin □ , Protease-26 ▽ .

methods appear to be sufficiently well correlated with the Farinograph technic to give a good estimate of activity in doughs. These methods include the Ayre-Anderson digestion of hemoglobin with Kjeldahl or spectrophotometric determinations, the bromsulphalein method, and the formol titration procedure. The Ayre-Anderson procedure was applicable to all the proteolytic preparations employed and the spectrophotometric modification makes this procedure fairly simple. The bromsulphalein method is both rapid and simple, and may be employed for most proteolytic preparations. When the optical density measurements are suitably transformed a linear relationship exists between enzyme concentration and optical density. The formol titration procedure, although very rapid and easy, was not suitable for the determination of preparations with low activity.

Acknowledgment

The financial assistance provided by Rohm and Haas Company, Philadelphia, is gratefully acknowledged. Acknowledgment is also made to the Rohm and Haas Company for the enzyme concentrates made available for this study, and to the Kansas Milling Company, Wichita, Kansas, for the malted wheat flour.

Literature Cited

1. Abbott, D. C., Miller, B. S., and Johnson, J. A. A spectrophotometric modification of the Ayre-Anderson method for estimation of proteolytic activity. Arch. Biochem. **38**: 85–89 (1952).
2. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.), Am. Assoc. Cereal Chem.: St. Paul, Minnesota (1947).
3. Ayre, C. A., and Anderson, J. A. Varietal differences in barleys and malts. VI. Autolytic proteolytic activity of malt and its correlations with wort nitrogen and barley nitrogen fractions. Can. J. Research **17C**: 239–246 (1939).
4. Balls, A. K., and Hoover, S. R. The milk-clotting action of papain. J. Biol. Chem. **121**: 737–745 (1937).
5. Fischer, E. H., and de Montmollen, R. Crystallization of the alpha-amylase of *A. oryzae*. Nature **168**: 606–607 (1951).
6. Greif, R. L. Use of bromsulphalein for the measurement of proteolytic activity. Proc. Soc. Exper. Biol. and Med. **75**: 813–815 (1950).
7. Hildebrand, F. C. A comparison of methods for the determination of proteolytic activity. Cereal Chem. **16**: 792–798 (1939).
8. Hildebrand, F. C. Comparison of methods for the determination of proteolytic activity. II. Studies on malted wheat flours. Cereal Chem. **17**: 635–639 (1940).
9. Hites, B. D., Sandstedt, R. M., and Schaumburg, Lorene. Study of proteolytic activity in wheat flour doughs and suspensions. II. A papain inhibitor in flour. Cereal Chem. **28**: 1–18 (1951).
10. Koch, F. C. Practical methods in biochemistry (3rd ed.), The Williams and Wilkins Co.: Baltimore, Md. (1941).
11. Koch, J. R., Nelson, O., and Ehrnst, L. Viscometer for routine determination of proteolytic activity of malts. Ind. Eng. Chem. Anal. Ed. **11**: 35–41 (1939).
12. Landis, Q., and Frey, C. N. A sensitive method for the determination of proteolytic activity. Cereal Chem. **15**: 91–101 (1938).
13. Johnson, J. A., and Miller, B. S. The relationship between dough consistency and proteolytic activity. Cereal Chem. **30**: 471–479 (1953).
14. Snedecor, G. W. Statistical Methods (4th ed.). Collegiate Press, Inc.: Ames, Iowa (1946).

EXTENSOGRAPH STUDIES OF STRUCTURAL RELAXATION IN BROMATED AND UNBROMATED DOUGHS MIXED IN NITROGEN[1]

C. J. Dempster, I. Hlynka, and J. A. Anderson

ABSTRACT

Structural relaxation of doughs containing 0, 20, 40, and 60 p.p.m. of potassium bromate has been investigated further with the extensograph technique previously described. Doughs were mixed in nitrogen to exclude the effect of oxygen of the air and to ensure that changes in relaxation curves were caused by the bromate.

Structural relaxation is exponential and is characterized by a rate constant. These rate constants increase slightly with time in unbromated doughs but decrease in bromated doughs; moreover, the rate constants change linearly with time. The higher the bromate concentration, the more pronounced is the time-dependent change in the rate constant. The slope of a plot of relaxation rate constant against reaction time provides a measure of the rate of the bromate reaction in dough for any given concentration.

The steady state load at the limit of decay of the structural relaxation curve is a function of the bromate concentration in dough and changes linearly with reaction time. In unbromated doughs, the steady state load decreases with time, while the reverse is observed in bromated doughs. An independent measure of the rate of the bromate reaction in dough is obtained from the relation between the steady state load and reaction time for any given bromate concentration. The rate is found to be directly proportional to the bromate concentration.

A previous publication (6) described a quantitative technique for using the extensograph to measure certain changes in the physical properties of doughs. Initial results were presented on what was termed the relaxation of internal stresses in dough, and on the effect of the action of bromate on the relaxation process.

Recent results by Smith and Andrews (11) and earlier work of other investigators (2, 8) suggested that the relaxation process might be affected by the oxygen of the air. The advisability of excluding oxygen when using the relaxation technique to study the kinetics of bromate reaction thus seemed to warrant consideration. Two objections suggested themselves: the necessity for building a fairly complex machine for mixing doughs in an inert gas; and the fact that the whole of the earlier investigation would have to be repeated. On the other hand, it appeared that these objections would be offset by the advantages of studying the bromate effect in as simple a system as

[1] Manuscript received May 18, 1953. Presented at the Annual Meeting of the American Association of Cereal Chemists, Dallas, Texas, April 20-24, 1952. Paper No. 129 of the Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Manitoba, and No. 309 of the Associate Committee on Grain Research (Canada).

possible. A suitable mixer was therefore built, and the earlier work on the effect of bromate on doughs mixed in air was repeated and expanded with doughs mixed in nitrogen.

As this and related investigations progressed, a need was felt for a more adequate terminology. It seemed necessary to distinguish more clearly between two types of relaxation. The first involves the relatively slow changes that occur in a resting dough after mixing or similar manipulation; these have been studied with the extensograph (6). The second involves the more rapid changes that occur through relaxation after an external stress has been imposed on a dough by stretching; these have been studied with the relaxometer (5, 9).

The first type was formerly referred to as "relaxation of internal stresses." It now seems more appropriate to refer to it as "structural relaxation." The intended implication is that changes occur in the structure of the dough. "Configurational elasticity" is an analogous term used by Alfrey (1) in describing other elastomers; but the use of such a specific term is not yet warranted in dough rheology.

A term is also required to characterize the high-energy state of the dough system after mixing; i.e., the reason why dough relaxes after it has been worked. For this purpose the term "activation," widely used in chemical kinetics, seems acceptable. It is consistent with descriptions used by the baker who refers to a freshly mixed or remixed dough as "excited" and "tighter."

Results presented in the following sections provide data on the effect on structural relaxation of bromate in the absence of oxygen. The study was made with unleavened dough and with the extensograph technique previously described (6).

Materials and Methods

The flour used in this study was milled in a Buhler laboratory mill from No. 1 Northern wheat to an extraction of about 70%. The protein content of the flour was 12.2% and the absorption was 61%.

Doughs were made with 200 g. of flour, water equivalent to 61% absorption, and 1% of sodium chloride. Potassium bromate in solution was added in specified amounts. Dough ingredients were temperature-conditioned overnight so that the doughs were taken from the mixer at a temperature of 30°C.

A special mixer was designed and constructed for those experiments in which doughs were mixed in nitrogen. This mixer (Fig. 1) is equipped with a bowl which makes an air-tight seal with the body of the mixer so that the mixing chamber can be evacuated. Three connections from the upper part of the mixing chamber lead 1) to a

manometer, 2) through a two-way stopcock to a Megavac vacuum pump or to a nitrogen source, and 3) to a separatory funnel by means of which the doughing liquid is added.

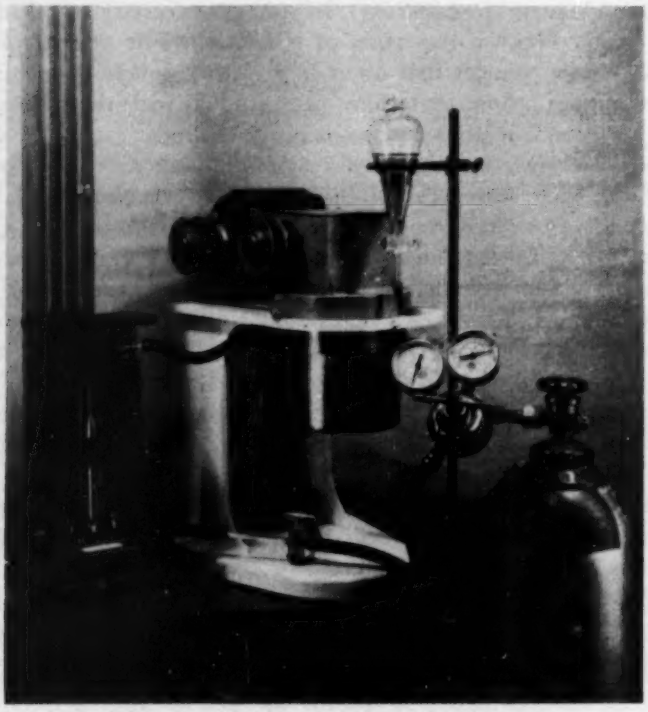

Fig. 1. Dough mixer set-up for mixing in nitrogen.

Prior to mixing, the flour samples are equilibrated in an atmosphere of nitrogen in an attempt to remove any adsorbed oxygen from the flour. Weighed flour samples in separate beakers are placed in a large desiccator which is evacuated to about 1.5 cm. of mercury by operating the Megavac pump for 2–2.5 minutes. The pressure in the desiccator is then restored to atmospheric by admitting nitrogen. The samples are kept in nitrogen overnight before being mixed.

The procedure used in mixing doughs in nitrogen is as follows. The 200-g. flour sample is placed in the mixing bowl, which is then evacuated to a pressure of about 2.5 cm. of mercury by operating the pump for about 1.5 minutes. After the vacuum pump is stopped, the doughing liquid is allowed to run quite rapidly from the separatory funnel into the mixing chamber. Pressure in the mixing chamber is

restored to atmospheric by admitting nitrogen. The mixer is started and mixing continued for 3 minutes. During mixing, a gentle stream of nitrogen is allowed to flow through the mixing chamber.

A Brabender Extensograph was used to measure structural relaxation in the doughs. The technique, which was based on earlier studies (6), may be outlined as follows: Flour doughs are prepared and allowed reaction times varying from 0 to 4 hours. The doughs are then shaped on the extensograph (i.e., rounded into a ball and rolled out into the test cylinder), clamped in dough holders, and set aside for varying rest periods. After the desired rest period, the dough is stretched by the extensograph and a load-extension curve is drawn. These extensograms are analyzed by reading the load supported by each dough at a constant sample deformation corresponding to a kymograph extension of 11 cm. (corrected). A correction is necessary to compensate for the downward displacement of the dough support during the stretching process. Accordingly, a small increment is added to the value 11 cm. to give the corrected kymograph extension at which the load is read. The correction is 0 cm. at 0 load and 1.44 cm. at 1000 g. load. The structural relaxation curve is obtained by plotting the extensogram load, at constant sample deformation, against rest period. Figure 2 illustrates the derivation of the structural relaxa-

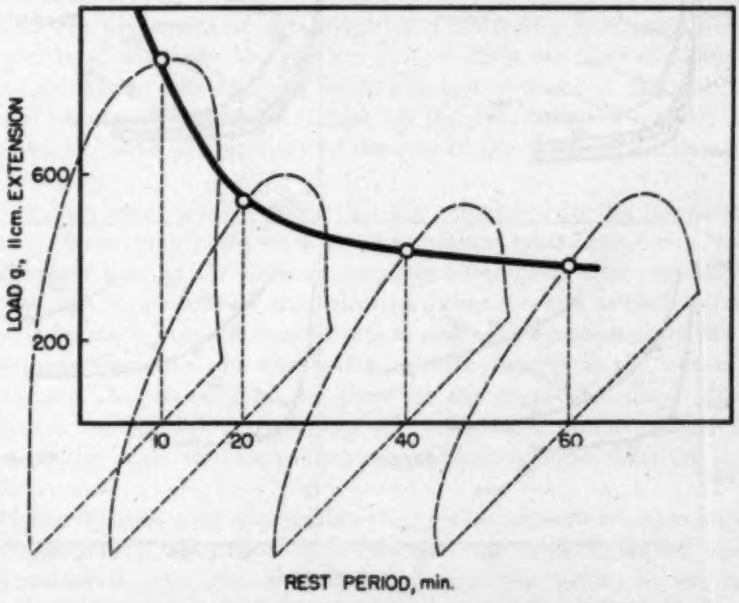

Fig. 2. Derivation of a structural relaxation curve from extensograms.

tion curve; the dashed curves represent extensograms obtained at different rest periods, and points representing the load at a constant sample deformation are joined to give the structural relaxation curve shown as a solid line.

Results and Discussion

Experiments were made on doughs containing 0, 20, 40, and 60 parts of potassium bromate per million parts of flour. Doughs of each bromate concentration were allowed reaction times of 0, 1, 2, 3, and 4 hours between the mixing and shaping operations.

Typical structural relaxation curves for these nitrogen-mixed doughs are shown in Fig. 3. The dashed curve in the upper left-hand section of Fig. 3 represents structural relaxation in unbromated doughs mixed in air and allowed no reaction time. This curve is included to give a comparison between structural relaxation in doughs mixed in nitrogen and doughs mixed in air. Elimination of oxygen of the air from dough by mixing in nitrogen has produced a decided downward shift in the structural relaxation curve. This shows that, to study

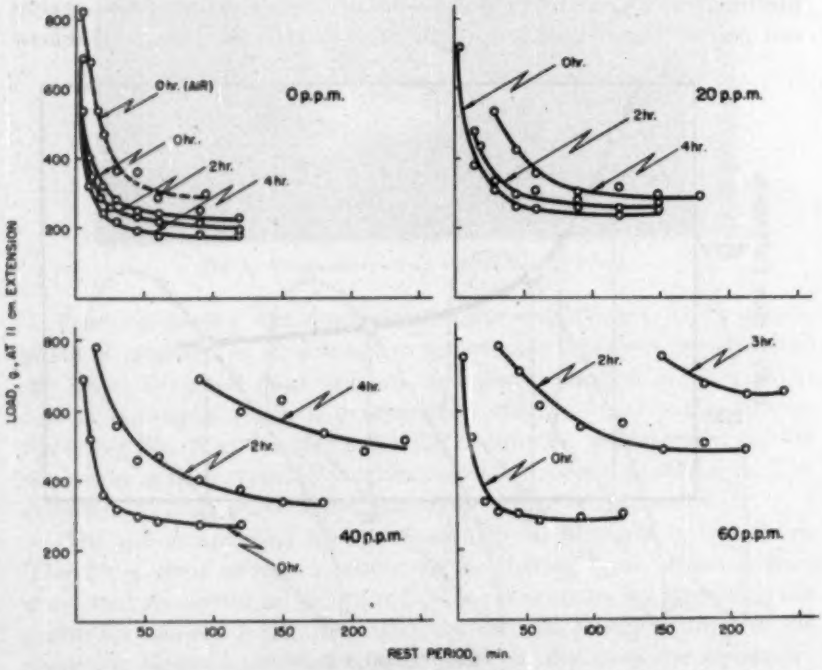

Fig. 3. Structural relaxation curves for nitrogen-mixed doughs containing 0, 20, 40, and 60 p.p.m. potassium bromate.

the effect on dough properties of bromate alone, oxygen must be eliminated from the dough.

The structural relaxation curves of Fig. 3 show that in general the results of experiments on the effect of bromate in doughs mixed in nitrogen parallel those previously presented (6) for bromated doughs mixed in air. For unbromated doughs, the structural relaxation curves are gradually displaced downward as reaction time is increased, i.e., the rate of decay gradually increases. Beyond the initial rapid decrease of load, there occurs a further slow, almost linear decrease of load. The incorporation of bromate produces a rather marked decrease in the rate of decay of extensogram load as reaction time is increased. The higher the bromate concentration, the more pronounced is this effect. Moerover, the load decays to a "steady state" value with increasing rest; i.e., the curve levels off. The steady state load increases with reaction time and bromate concentration.

From primary data such as the structural relaxation curve, quantitative information about the reaction of bromate in dough may be derived by an extension of the analytical treatment suggested in the previous work (6). The progressive changes in dough properties evident in the structural relaxation curves can be assessed quantitatively by observing the changes in the structural relaxation rate constants with reaction time. If the structural relaxation rate constant summarizes the properties of the dough at a particular reaction time—i.e., the time when the doughs are shaped—then the rate of change of the relaxation rate constant is, in effect, a measure of the rate of change of dough properties caused by the reaction of bromate; it may also be taken as a measure of the rate of the bromate reaction in dough.

The rate constants for relaxation are calculated in the following manner. From each point on a given structural relaxation curve, the steady state load at the limit of decay is subtracted. The regression line for the logarithms of the resulting values vs. rest period is calculated for each bromate concentration and each reaction time. The structural relaxation rate constants are then derived as the product of 2.3 and the negative of the slope of the regression lines. This analytical method of determining the relaxation rate constants is more precise than the approximate graphical method used in our previous work.

Figure 4 shows that appreciable changes occur with reaction time in the structural relaxation rate constants for both bromated and unbromated doughs. For unbromated doughs the values of the relaxation rate constants exhibit a considerable scatter, but in general

structural relaxation becomes more rapid as reaction time is increased. For bromated doughs, the relaxation rate constants are found to decrease with time—the higher the concentration, the more rapid the decrease. For a concentration of 20 p.p.m. of bromate, the relaxation rate constants decrease linearly with increasing reaction time beyond about 1 hour. For higher bromate levels, the exact relation is less clear.

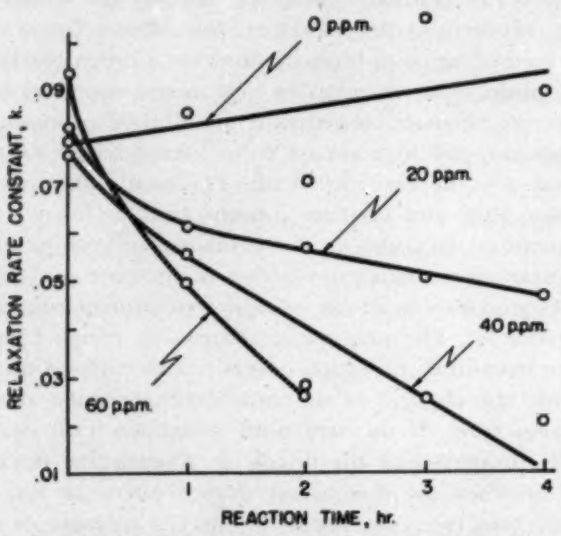

Fig. 4. Effect of reaction time on structural relaxation rate constants for nitrogen-mixed bromated and unbromated doughs.

The slope of any of the curves of Fig. 4 is a measure of the rate of change in dough properties for the bromate concentration specified and may be taken as a measure of the rate of bromate reaction in dough at that concentration. For purposes of further discussion an approximation, that the stabilized portion of the curves beyond about the first hour may be regarded as linear, has been made. Slopes for these curves obtained in this manner are recorded in Table I.

An independent estimate of the rate of bromate reaction can be obtained by making use of the steady state load values at the limit of decay of the structural relaxation curves. The steady state load value is regarded as an equilibrium value (for this particular rate of extension of dough in the extensograph test) of the dough properties which result from the reaction of a specified amount of bromate for a particular reaction time. Figure 5 shows a plot of the steady state load value against reaction time for the various bromate concentra-

TABLE I

RATES OF BROMATE REACTION IN DOUGH

Bromate Concentration	Rate of Bromate Reaction (Arbitrary Units)	
	Calculated from Relaxation Rate Constants	Calculated from Steady State Loads
p.p.m.		
0	+ 2.59	− 11.2
20	− 4.74	+ 18.3
40	−14.0	+ 73.3
60	−22.9	+140.0

tions. For unbromated doughs, the load value plotted is that at which the relaxation curve appears to show a change from the initial exponential decay to a slower, almost linear decay of load.

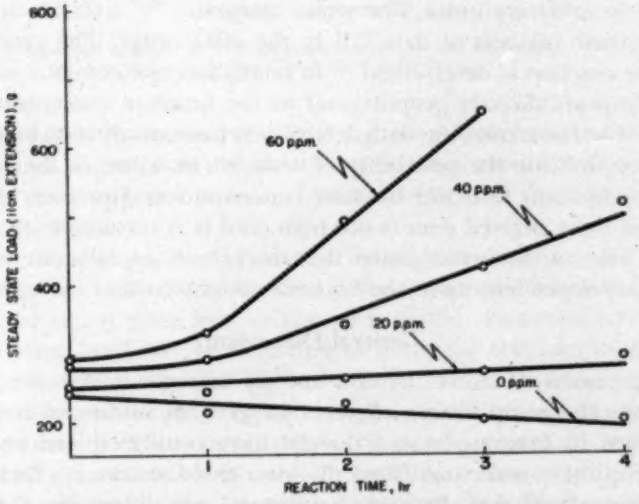

Fig. 5. Effect of reaction time on steady state or final load supported by nitrogen-mixed doughs.

For each bromate concentration, the steady state load values appear to change linearly as reaction time is increased beyond 1 hour. For zero bromate the curve is of negative slope, whereas for the various bromate concentrations used the curves are of positive slope. The higher the bromate concentration, the more rapid is the change in load with time. There is a slight curvature in these plots for the higher bromate concentrations in the range of 0 to 1 hour reaction time. An induction period in the reaction of bromate in dough may

be responsible for the observed curvature. In addition, the increase in the steady state load value with bromate concentration at zero reaction time indicates that bromate may have an immediate effect in dough, as suggested by some of the results of Smith and Andrews (11).

The steady state load is a measure of dough properties at a given time. The rate of change of the steady state load for each bromate concentration will then be taken as a measure of the rate of the bromate reaction in dough. The slopes of the stabilized portions of the above curves as a measure of the rate of the bromate reaction in dough are recorded in Table I, along with the reaction rates calculated from the relaxation rates.

Figure 6 shows the relation between the rate of the bromate reaction in dough, as estimated by these two methods, and the bromate concentration in the dough. The rates of reaction being plotted are, of course, the calculated slopes of the curves of Figs. 4 and 5 expressed in arbitrary units. The scales along the "y" axis are adjusted so that these two sets of data fall in the same range. The rate of the bromate reaction as determined from relaxation rate constant measurements appears directly proportional to the bromate concentration in dough. The corresponding data determined from steady state load measurements indicate the possibility of a slight curvature in the relation between reaction rate and bromate concentration. However, the precision of these derived data is not high, and it is reasonable to assume at this stage of the investigation that the rate of the bromate reaction is linearly dependent upon the bromate concentration in dough.

General Discussion

The results obtained by the use of nitrogen-mixed doughs (to eliminate the complicating effect of oxygen) in studies of structural relaxation in bromate-treated doughs have confirmed and extended the preliminary work published (6) from this Laboratory. Previously, it was postulated that the rate of structural relaxation, i.e., the structural relaxation rate constant, was determined by the extent of cross linking in the network structure of dough and that changes in the relaxation rate constant indicated changes in the degree of cross linking. It seems logical to extend the interpretation of the results of structural relaxation studies by regarding the rate of change of the structural relaxation rate constant with reaction time as a measure of the rate of cross linking produced in dough by a given concentration of potassium bromate. Thus the slopes of the stabilized portions of the curves of Fig. 4 are regarded as measures of the rate of cross

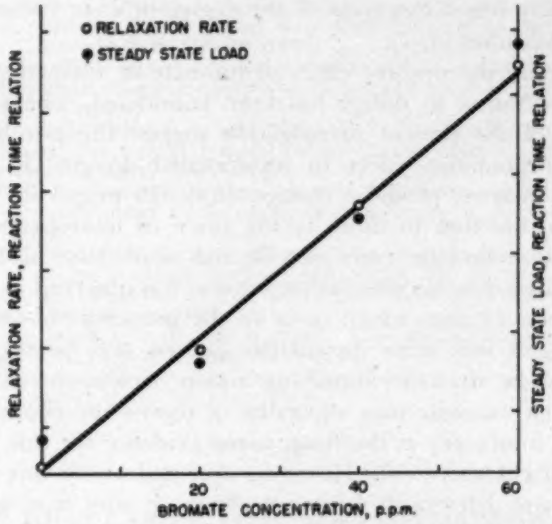

Fig. 6. Comparison of effect of bromate concentration on rate of bromate reaction in dough by two independent methods.

linking caused by the reaction in dough of the various concentrations of bromate. Figure 6 then shows that the rate of cross linking in dough is to a close approximation a linear function of the bromate concentration.

The same molecular picture of dough structure may be used to interpret the independent results of the structural relaxation studies using the steady state load at the limit of the relaxation curve. The steady state load may be regarded as reflecting the condition which was previously postulated (6) for a completely relaxed dough, i.e., an equilibrium between the number of labile cross links breaking and re-forming. The magnitude of the steady state load may be regarded as a function of the average number of these bonds (or alternately a measure of the strength of the bonds); and changes in the steady state load resulting from the reaction of bromate may be regarded as due to the introduction of additional cross links in the dough structure.

This interpretation of steady state load is strengthened by somewhat analogous developments in the field of high polymer chemistry. Equilibrium swelling volume of polymers has been related through an equation of state (3) to the tension or force of retraction exhibited by a stretched polymer. Published experimental data indicate that both the equilibrium swelling volume and the force of retraction are

approximately linear functions of the degree of cross linking or network concentration (4, 7).

So far, only the positive effect of bromate in increasing the network concentration in dough has been considered. The analysis of the results of the present investigation suggest the possibility of a secondary degradative effect in unbromated doughs. It has been shown that bromate produces changes in dough properties which are opposite in direction to those taking place in unbromated doughs, i.e., structural relaxation rates increase and steady state loads decrease in unbromated doughs, whereas the reverse was observed in bromated doughs. These changes which occur in the properties of unbromated doughs suggest that some degradative process may be taking place. It may thus be that the improving action of bromate in dough is twofold. First, bromate may neutralize or repress the effects of native deleterious substances in the flour. Some evidence for this possibility is presented elsewhere (10). However, over and above this repression of the effect of deleterious substances, bromate must have some other positive effect in the dough, since the incorporation of bromate not only eliminates but reverses the direction of changes in dough properties. There is an alternate possibility that the degradative process is not reversed by bromate and that two opposing reactions—the degradative reaction and the reaction of bromate—take place simultaneously. The positive changes in dough properties as a result of the reaction of bromate may completely overshadow the effects of a degradative reaction. Whether or not the degradative process may be identified with proteolysis can only be answered by further studies.

The essentials of the hypothesis previously advanced to explain the observed behavior of dough remain unchanged with this extension of structural relaxation studies. Briefly, the hypothesis is as follows: the behavior of dough is attributed to the existence of a three-dimensional or network structure, the cross links of which are labile bonds between polar groups of adjacent molecules; bromate progressively creates potentially reactive groups in certain molecules in the dough which interact to form additional nonlabile cross links between adjacent molecules only when brought together when the dough is worked.

Literature Cited

1. Alfrey, T., Jr. Mechanical behavior of high polymers. Interscience Publishers, Inc.: New York (1948), p. 103.
2. Baker, J. C., and Mize, M. D. Mixing doughs in vacuum and in the presence of various gases. Cereal Chem. 14: 721–734 (1937).
3. Bardwell, J., and Winkler, C. A. The formation and properties of three-dimensional polymers. I. Statistics of network polymers. Can. J. Research 27B: 116–127 (1949).

4. BARDWELL, J., and WINKLER, C. A. The formation and properties of three-dimensional polymers. III. The effect of network structure on elastic properties. Can. J. Research **27B**: 139–150 (1949).
5. CUNNINGHAM, J. R., HLYNKA, I., and ANDERSON, J. A. An improved relaxometer for viscoelastic substances applied to the study of wheat dough. Can. J. Tech. **31**: 98–108 (1953).
6. DEMPSTER, C. J., HLYNKA, I., and WINKLER, C. A. Quantitative extensograph studies of relaxation of internal stresses in non-fermenting bromated and unbromated doughs. Cereal Chem. **29**: 39–53 (1952).
7. FLORY, P. J., RABJOHN, N., and SHAFFER, M. C. Dependence of elastic properties of vulcanized rubber on the degree of cross linking. J. Polymer Sci. **4**: 225–245 (1949).
8. FREILICH, J., and FREY, C. N. Dough oxidation and mixing studies. VII. The role of oxygen in dough mixing. Cereal Chem. **24**: 436–448 (1947).
9. HLYNKA, I., and ANDERSON, J. A. Relaxation of tension in stretched dough. Can. J. Tech. **30**: 198–210 (1952).
10. HLYNKA, I., TEMPLIN, P. R., and ANDERSON, J. A. Decomposition of bromate in dough. Cereal Chem. **30**: 391–403 (1953).
11. SMITH, D. E., and ANDREWS, J. S. Effect of oxidizing agents upon dough extensograms. Cereal Chem. **29**: 1–17 (1952).

IS THE HEAT-LABILE LOAF VOLUME DEPRESSANT OF MILK A PROTEASE OR PROTEASE ACTIVATOR?[1, 2]

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ABSTRACT

The heat-labile loaf volume depressant factor in unheated milk serum proteins is neither a protease nor an activator of flour proteases. Unheated milk serum protein preparations reduced the viscosity of sols of gluten in acetic acid, but this appeared to be due to suppression of the electroviscous effect by traces of salts rather than to proteolysis. Furthermore, at pH values likely to be encountered in dough, the serum proteins did not liberate non-protein nitrogen from wheat gluten or hemoglobin and did not accelerate the action of flour proteases on the latter. Moreover, a fraction of the serum proteins ("lactoglobulin"), which had been shown previously to be especially rich in the loaf-volume depressant, failed to liberate tyrosine or tryptophan from hemoglobin at pH 5.7.

In previous papers (3, 5) it was reported that the heat-labile loaf volume depressant of the serum proteins of milk resides in the so-called "lactoglobulin" fraction (proteins precipitated at 43% saturation with ammonium sulfate) of that mixture and that the depressing effect probably is not the result of the action of protein sulfhydryl groups. It appeared possible that the loaf-volume depressant may be

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[2] The contents of this paper constitute part of a thesis submitted by Arthur L. Gordon to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree, August 1952.
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either a protease or an activator of flour proteases and that its action involves a proteolytic attack on the gluten matrix.

Skovholt and Bailey (11) were unable to demonstrate any increase in non-protein nitrogen in fermenting dough as a result of incorporating unheated milk, but they recognized that their methods of separation (precipitation by copper salts or tungstic acid) were not sufficiently sensitive to detect small degrees of proteolysis.

The meager literature on the proteases of milk (7) indicates that they are precipitated with casein when milk is fractionated and thus would not be expected to be present in serum proteins. The most adequately defined milk protease is that found by Warner and Polis (13) in casein. This enzyme has maximum activity at pH 9.2.

In spite of the fact that available literature indicates the absence of proteases from milk serum proteins, it was considered worthwhile to reinvestigate the problem. The study was given further impetus because the possibility that milk serum proteins contain an activator of flour proteases has never been precluded, although Ashworth and Harland (2) found that milk not only failed to activate papain in its action on gluten but that it also inhibited the activation of papain by yeast.

In the present study the viscosimetric technique employed by Olcott *et al.* (9) to demonstrate the effects of proteases and of reducing agents on acetic acid dispersions of gluten was employed in the search for proteases in milk serum protein and its fractions. Unfortunately this technique is limited in its application since sols of gluten in acetic acid are not stable at pH values much above 4.0. As other indices of proteolysis, non-protein nitrogen analyses and the Folin-Ciocalteau procedure were used.

Materials and Methods

The flour employed in the enzymatic studies and for the preparation of gluten was of the same hard red spring wheat type previously used in baking studies (3). Sols of gluten in acetic acid were prepared essentially according to the procedure of Lusena (6).

Gluten was prepared by mixing a flour-water dough in a mechanical mixer and then washing with hand kneading in a fine stream of 0.1% sodium chloride solution. The washed gluten was dispersed in 0.05 *N* acetic acid by slowly homogenizing in a Waring Blendor, the speed of which was controlled by a variable transformer. The sol was allowed to stand for at least 1 hour at 5°C. to permit entrapped air to escape. It was then slowly titrated with saturated calcium hydroxide solution to pH 6.5; the gluten appeared as flocs which aggregated to

form an elastic mass. After 1 hour the milky supernatant, containing starch, was siphoned off, and the gluten washed twice with distilled water and dispersed in 0.025 *N* acetic acid. Finally this sol was centrifuged for 15 minutes at 1500 r.p.m. in an International Centrifuge (No. 2), the precipitate being discarded. Such sols have a pH of approximately 4. They were heated, when desired, by running through a glass coil immersed in a boiling-water bath; the treatment amounted to approximately 7 minutes at 95°C.

Casein, milk serum proteins, and the "lactoglobulin" and "lactalbumin" fractions were prepared as previously described (3).

The hemoglobin used in certain experiments was Armour's "bovine hemoglobin enzyme substrate powder"; that employed in others was prepared in this laboratory and kindly furnished by Dr. I. E. Liener.

Measurements of viscosity were made on dispersions of gluten in 0.025 *N* acetic acid containing 2-4 mg. of gluten nitrogen per ml. Milk protein fractions or other materials being studied were dispersed in these sols. A ratio of milk serum protein to gluten of 1:2 was used in order to obtain significant changes, although it is obviously much higher than the ratio of 1:20 to 1:25 in a dough supplemented with 6% milk solids. In certain experiments glutathione was employed at a concentration of 0.01 *M* which furnishes about 25 times as many sulfhydryl groups per unit of gluten as the serum proteins in milk-supplemented dough. In other tests papain at a level of 0.02% was used. The mixture was introduced into an Ostwald pipette, tempered to 25°C. in a water bath, and the viscosity measured at appropriate intervals. The data are expressed as specific viscosity, η_{sp}, which equals

$$\frac{\eta \text{ sol}}{\eta \text{ solvent}} - 1.$$

The determination of proteolysis in terms of non-protein nitrogen was made in two different series of experiments. In the first, dispersions of gluten and milk protein fractions or other source of enzyme in 0.025 *N* acetic acid were incubated at 25°C. for the desired length of time. The proteins were precipitated by adding sufficient 16% trichloracetic acid to give a final concentration of 12% and were filtered off on Whatman No. 2 paper. Nitrogen in the filtrate was determined by the microkjeldahl procedure (10). In the second series of experiments the Miller (8) modification of the Ayre-Anderson method of determining proteolysis in flour was employed. Mixtures of flour or bovine hemoglobin substrate (Armour's) or both and the appropriate milk protein fraction were prepared in buffered solutions with pH

values ranging from 3.6 to 6.4. The mixtures contained 5 g. of flour, 0.6 g. of hemoglobin, 25 mg. of serum protein (or 10 mg. of "lactoglobulin") in 25 ml. of buffer. Acetate buffers of pH 3.68, 4.30, and 4.8 and phosphate buffers of pH 5.70 and 6.36 were employed. All had an ionic strength of 0.1. Duplicate samples were prepared in each case, one being precipitated after 5 minutes with trichloracetic acid (final concentration = 10%), the other being incubated at 30° or 40°C. for 3 or 5 hours before similar treatment. The samples were filtered through Whatman No. 2 paper and nitrogen was determined in the filtrate by the microkjeldahl method.

In experiments in which proteolysis was determined by the liberation of phenol and indole groups, the methods of Anson (1) were employed. Washed hemoglobin was used as substrate either with or without urea denaturation ("trypsin-papain" and "pepsin" substrates respectively). In both cases the substrate was equilibrated by dialysis against a phosphate buffer of pH 5.7, $\mu = 0.1$. To 5 ml. of 2% hemoglobin in the phosphate buffer, 1 ml. of dispersion of the protein was added (containing 10 mgm. of "lactoglobulin" or 30 mgm. of casein), to be tested for enzyme activity. Duplicate samples were prepared, one being treated with 10 ml. of 0.3 *N* trichloracetic acid immediately and the second after incubation at 30°C. for 3 hours. Precipitated protein was removed by twice filtering through Whatman No. 2 paper. To 5 ml. of the filtrate was added 10 ml. of 0.5 *N* sodium hydroxide solution and 3 ml. of Folin-Ciocalteau phenol reagent (1 part stock solution diluted with 2 parts water). After 10 minutes the color intensity was determined at 640 $m\mu$ with a Coleman Model 11 spectrophotometer.

Results

Effects on Viscosity of Gluten Sols. In Fig. 1 are shown the effects of heat treatment, of added milk serum protein, and of papain on the viscosity of gluten sols containing 2 mg. of gluten nitrogen per ml., and the effect of glutathione on a sol containing 4.1 mg. nitrogen per ml. during incubation periods up to 4.5 hours at 25°C. As expected from the report of Olcott *et al.* (9), unheated gluten exhibited a progressive decrease in viscosity and heat treatment abolished this effect. The addition of milk serum protein at a concentration one-half that of the gluten produced a markedly lower viscosity from the outset. This effect is quite unlike that of papain (0.02%), which produced a gradual diminution of viscosity. The effect of glutathione (0.01 *M*) also differed markedly from that of the milk serum proteins.

The reduction of viscosity of gluten sols by milk serum proteins is

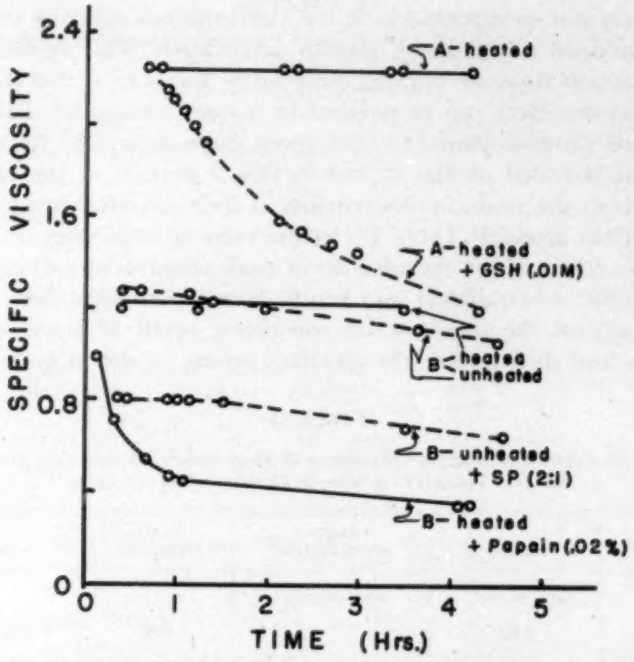

Fig. 1. Effects of heat treatment, milk serum proteins, glutathione, and papain on the viscosity of sols of gluten in acetic acid upon incubation for various times at 25°C. The curves, reading from top to bottom, give results for the following systems: Curves 1 and 2: Gluten sol A (4.1 mg. N/ml.)—(1) heated; (2) heated, plus glutathione (GSH) at a final concentration of 0.01 M (0.3%). Curves 3, 4, and 5: Gluten sol B (2.0 mg. N/ml.) — (3) heated and unheated; (4) unheated, plus milk serum protein (SP) at a concentration of 1.0 mg. N/ml.; (5) heated, plus papain (0.02%).

TABLE I

The Effect of Milk Serum Proteins and their Ash on the Viscosity of Gluten Sols in Acetic Acid

Experiment No.	Specific Viscosity[1] at 25°C. and 20 min. after mixing				
			Gluten + Serum Protein[2,3]		
	Gluten Control[2]	Serum Protein Control[3]	Serum Protein Partially Dialyzed	Serum Protein Exhaustively Dialyzed	Gluten + Ash[4] of Serum Protein
1	1.23	....	0.69	1.01	....
2a	1.62	0.03	....	1.19	1.41
2b	1.62	0.03	....	1.04	1.25
3	1.56	0.06	....	1.20	1.44

[1] Determined with Ostwald pipettes; outflow time for water about 40 sec.
[2] Gluten concentration 2 mgm. N/ml.; unheated gluten used in experiments 1 and 3, heated in 2a and 2b.
[3] Serum protein concentration 0.5 mgm. N/ml.
[4] Ash from an amount of serum protein equivalent to 0.5 mgm. N/ml.

very likely due to suppression of the electroviscous effect by traces of salts contained in the serum protein preparation. This argument receives support from the findings recorded in Table I: 1) that the magnitude of the effect can be reduced by a more exhaustive dialysis of the serum proteins than had been given the sample used for the experiment recorded in Fig. 1; and 2) that a portion of the effect of exhaustively dialyzed samples remains in their ash after incineration. It is further attested (Table II) by the extreme sensitivity of sols of gluten in acetic acid to the addition of small amounts of sodium chloride. Finally, when gluten and serum protein are separately equilibrated against the same solvent containing acetic acid and sodium chloride and then mixed, the specific viscosity of the mixture is ap-

TABLE II

THE EFFECTS OF SODIUM CHLORIDE AND MILK SERUM PROTEINS ON THE SPECIFIC VISCOSITY OF SOLS OF GLUTEN IN ACETIC ACID

Sol	Gluten[1]	Milk Serum Protein	Sodium Chloride	η_{sp}
	mg. N/ml.	*mg. N/ml.*	%	
1	2.42	....	0.00	1.62
	2.42	....	0.05	1.06
	2.42	....	0.075	0.88
	2.42	....	0.10	0.70
	2.42	....	0.15	0.55
	2.42	....	0.20	0.47
	2.42	....	0.25	precipitated
2	2.54	....	0.00	1.85
	2.54	....	0.175	0.41
	2.54	....	0.200	0.39
	2.54	....	0.225	precipitated
3	a 2.54	....	0.200	0.39
	b	0.8	0.200	0.02
	c 1.27[2]	....	0.200	0.16
	d ....[3]	0.4	0.200	0.01
	e 1.27[4]	0.4	0.200	0.18

[1] Heated gluten sols.
[2] Mixture of equal parts a and solvent.
[3] Mixture of equal parts b and solvent.
[4] Mixture of equal parts a and b.

TABLE III

NON-PROTEIN NITROGEN PRODUCED BY INCUBATING MIXTURES OF GLUTEN WITH MILK SERUM PROTEIN OR PAPAIN AT 25°C.

System[1]	Time of Incubation	Non-Protein Nitrogen
	Min.	*mg./100 ml.*
Gluten control	17	3.0
	78	4.0
	226	4.0
Gluten + unheated milk serum protein[2]	21	5.0
	79	5.0
	219	9.0
Gluten + milk serum protein[2] heated 78°C. for 30 min.	18	5.0
	79	5.0
	222	5.0
Gluten control	15	0.7
	46	2.0
	235	0.7
Gluten + cysteine hydrochloride (0.0002 *M*)	15	2.0
	52	2.0
	253	2.0
Gluten + papain (0.02%)	18	6.7
	48	8.7
	252	22.1
Gluten + papain + cysteine hydrochloride	15	7.4
	49	11.4
	241	24.0
Gluten + cysteine hydrochloride + papain heated 15 min. at 70°C.	15	3.4
	41	3.4
	248	5.3

[1] Gluten concentration 2 mg. N/ml. Heated gluten used.
[2] Milk serum protein concentration of 0.5 mg. N/ml.

proximately the summation of those of the separate components (Table II, sol 3). This indicates that no proteolysis or other interaction occurred. The viscosity measurements do not indicate the presence of proteases in milk serum protein capable of attacking gluten at pH 4.0.

Liberation of Non-Protein Nitrogen. Table III shows the effect of incubating milk serum proteins or papain (with the inclusion in some instances of cysteine as an activator) with gluten in acetic acid solution. There is only a slight indication of a proteolytic effect by the unheated serum protein, whereas papain, as expected, produced significant quantities of non-protein nitrogen.

The more extensive study recorded in Fig. 2, in which flour, hemoglobin, and milk serum proteins in various combinations were incu-

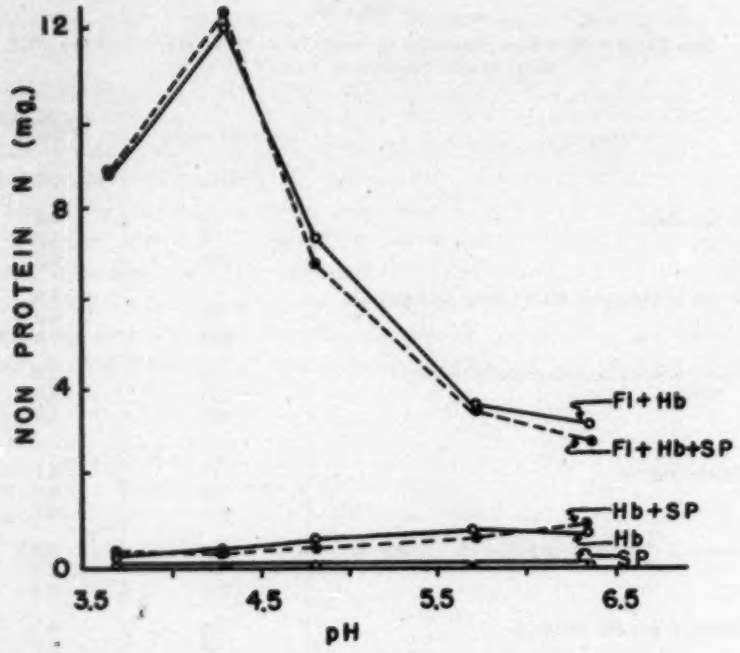

Fig. 2. Liberation of non-protein nitrogen from various flour and protein systems upon incubation at various pH values for 3 hours at 40°C. Fl = flour, 5 g.; Hb = hemoglobin, 0.6 g.; SP = serum protein, 25 mg. Each system contained 25 ml. of buffer.

bated at various pH values, gives no evidence for a protease in the serum proteins. The flour proteases were active, with an optimum pH in the neighborhood of 4.2, but were not further activated by the presence of milk serum proteins. Evidently serum protein does not contain an activator of flour proteases.

An analogous study with acid-precipitated casein over a pH range from 3.68 to 6.85 also failed to demonstrate the presence of any protease or activator therein. Although casein is known to contain a protease active at high pH, insufficient proteolysis occurred under the conditions of this experiment to be detected by non-protein nitrogen determinations.

The "lactoglobulin" fraction, which has been found to be rich in loaf-volume depressant (3), was investigated for protease activity. Incubation of 5 g. of flour and 0.6 g. of hemoglobin for 3 hours at 30°C. in 25 ml. of buffer at pH 5.7 produced 3.9 mg. of non-protein nitrogen. Inclusion of 10 mg. of "lactoglobulin" in the mixture increased the non-protein nitrogen to 4.1 mg., a relatively insignificant difference.

TABLE IV

PROTEOLYTIC ACTION OF "LACTOGLOBULIN" AND CASEIN ON HEMOGLOBIN SUBSTRATES AS MEASURED BY THE FOLIN-CIOCALTEAU REAGENT

Preparation[2]	Optical density[1] at 640 mμ		Apparent Tyrosine Liberated by Incubation
	Not Incubated	Incubated[3]	
			mg./g. hemoglobin
"Lactoglobulin" control	0.088	0.082	nil
Casein control	0.079	0.060	nil
Hemoglobin substrate,			
not denatured			
Hemoglobin alone	0.018	—	—
Hemoglobin + "lactoglobulin"	0.074	0.082	<0.04
Hemoglobin + casein	0.035	0.032	nil
Hemoglobin substrate,			
denatured			
Hemoglobin alone	0.035	0.035	nil
Hemoglobin + "lactoglobulin"	0.088	0.086	nil
Hemoglobin + casein	0.055	0.135	0.64

[1] 13-mm. cells in Coleman Model 11 Spectrophotometer.
[2] "Lactoglobulin" preparations contained 10 mgm./ml. and casein preparations contained 30 mgm./ml.
[3] Incubation at 30°C. and pH 5.7 for 3 hours.

Liberation of Tyrosine and Tryptophan. In Table IV are shown results of the tests for protease in "lactoglobulin" and casein in which hemoglobin was the substrate and liberated phenol and indole groups were determined with the Folin-Ciocalteau reagent. This method is sensitive enough to show a significant action of the protease of casein on the denatured hemoglobin but does not reveal any action by "lactoglobulin."

Discussion

These experiments demonstrate that the heat-labile loaf-volume depressant of milk serum proteins is not a protease. Obviously they do not preclude the possible presence of a protease or protease activators under conditions different from those employed in this study. However, since the pH, temperature, and time used somewhat simu-

late the conditions in fermenting bread dough, it is quite unlikely that any milk serum protease is active in the latter. Furthermore, milk serum proteins appear not to activate the proteases of flour.

The depression of loaf volume and slackening of dough by reducing agents has been attributed to a direct action on flour proteins (12) or to an activation of flour proteases (4). Apparently the action of the milk serum protein factor differs from either of these mechanisms.

Acknowledgments

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Literature Cited

1. Anson, M. L. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. J. Gen. Physiol. **22:** 79–89 (1938).
2. Ashworth, U. S., and Harland, H. A. Chemical factors affecting the baking quality of dry milk solids. II. The effect of milk on gluten fractionation. Cereal Chem. **19:** 830–837 (1942).
3. Gordon, A. L., Jenness, R., and Geddes, W. F. Further studies of the heat-labile loaf volume depressant of milk serum proteins. Cereal Chem. **30:** 213–221 (1953).
4. Jørgensen, H. On the existence of powerful but latent proteolytic enzymes in wheat flour. Cereal Chem. **13:** 346–355 (1936).
5. Larson, B. L., Jenness, R., and Geddes, W. F. The effect of various milk serum proteins and their sulfhydryl groups on bread quality. Cereal Chem. **29:** 440–447 (1952).
6. Lusena, C. V. Preparation of dried native wheat gluten. Cereal Chem. **27:** 167–178 (1950).
7. McMeekin, T. L., and Polis, B. D. Milk Proteins. Adv. Prot. Chem. **5:** 201–228 (1949).
8. Miller, B. A critical study of the modified Ayre-Anderson method for the determination of proteolytic activity. J. Assoc. Offi. Agr. Chemists **30:** 659–669 (1947).
9. Olcott, H. S., Sapirstein, L. A., and Blish, M. J. Stability of wheat gluten dispersions toward reducing agents in the presence and absence of a gluten proteinase. Cereal Chem. **20:** 87–97 (1943).
10. Pepkowitz, L. P., and Shive, J. W. Kjeldahl nitrogen determination. A rapid wet-digestion micromethod. Ind. Eng. Chem., Anal. Ed. **14:** 914–916 (1942).
11. Skovholt, O., and Bailey, C. H. The effect of mixing and fermentation upon the protein structure and colloidal properties of doughs. Cereal Chem. **12:** 307–320 (1935).
12. Sullivan, Betty. The mechanism of the oxidation and reduction of flour. Cereal Chem. **25** (suppl.): 15–31 (1948).
13. Warner, R. C., and Polis, Edith. On the presence of a proteolytic enzyme in casein. J. Am. Chem. Soc. **67:** 529–532 (1945).

COMPOSITION OF THE PENTOSANS EXTRACTED BY ACID FROM WHEAT: ITS RELATIONSHIP TO MILLING BEHAVIOR[1]

W. S. Hale, Ali Mohammad, and D. K. Mecham

ABSTRACT

In previous work with Pacific Northwest soft wheats, an inverse correlation was found between their milling properties and the amounts of pentosan extracted from them with dilute acid. Xylose and arabinose now have been separated by paper chromatography after hydrolysis of the 2 *N* hydrochloric acid extracts (6 hours' extraction at 25°C.) of wheats of the same type, and the individual pentoses determined quantitatively. Ratios of arabinose to xylose ranged from 0.6 to 1.7. With 30 samples, representing ten varieties grown at three stations, the amounts of xylose extracted were inversely correlated within stations with both straight flour yield and a milling score (r values, −0.70* to −0.95**). The amounts of arabinose did not show a consistent relationship to milling behavior (r values, +0.36 to −0.81**). The sums of xylose and arabinose were as consistently related to the milling values as xylose alone, however (r values, −0.65* to −0.95**).

Bran containing relatively little endosperm was separated in a rice-milling machine from four of the wheats. When extracted with 2 *N* hydrochloric acid, these bran preparations yielded three to five times more arabinose than xylose. Therefore, pentosans extracted from the endosperm rather than from bran appear predominantly responsible for the correlations found with extracts of whole wheats.

In an effort to relate variations in milling behavior of wheats to differences in their composition, attention has been directed to the pentosan constituents of the wheat kernel. In a previous report (3), procedures were described for extraction of part of the pentosans present in ground wheat by a mild acid treatment and for determination by an orcinol method of the amounts of pentosans so extracted. When these procedures were applied to soft wheats from the Pacific Northwest area, a highly significant, inverse correlation was obtained between the amounts of extracted pentosans and either flour yield or a milling score of the wheats.

The methods used in the foregoing work were developed largely in an empirical way. However, Perlin (10, 11), investigating the nature of the water-soluble pentosans present in wheat flour, found the major portion of these pentosans to have a characteristic basic structure of a straight chain of xylose residues to which arabinose branches,

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limited to one residue in length, were attached. Thus, the way in which the xylose residues contributed to the physical properties of the pentosans was quite different from that in which the arabinose residues contributed. If the acid-extractable pentosans are assumed to be similar in structure to the water-soluble pentosans, milling behavior might be more closely related to the amounts of one of the pentoses than to the amounts of the other. To investigate this possibility, the xylose and arabinose contents of acid extracts of several wheats differing in milling behavior have been determined. In addition, with four wheats, xylose and arabinose contents of water and acid extracts of whole wheats and acid extracts of bran preparations containing relatively little endosperm have been compared.

Materials and Methods

The Pacific Northwest soft wheat samples were obtained through the cooperation of the Western Wheat Quality Laboratory, U. S. Bureau of Plant Industry, Soils, and Agricultural Engineering, located in Pullman, Washington, which also supplied the milling data, obtained with a Buhler experimental mill (12, 13). The moisture content of these wheat samples was consistently near 10% when received.

To obtain bran samples more nearly free of endosperm than those produced on an experimental mill, the following procedure was used. Broken kernels were removed from the wheat samples; then 6 to 7 ml. of water per 100 g. wheat were added with thorough mixing, and the moistened wheat was allowed to stand 10 minutes. The wheat samples then were placed in a McGill experimental rice miller[2] and, with the application of light pressure, bran coatings were removed from the wheat kernels. The bran so obtained was dried at room temperature and then carefully picked over by hand to remove fragments of kernels. Yields of about 5 g. bran per 100 g. wheat were obtained. Starch analyses made on four preparations, by the method of Hoffpauir (4), gave values of 1.3, 1.2, 3.5, and 1.6%; the preparations, therefore, were relatively free of endosperm.

Preparation of Extracts: Pentosans were extracted by treatment with 2 *N* hydrochloric acid at 25°C. for 6 hours; these conditions were used because they were observed by Elder *et al.* (3) to extract amounts of pentosans correlated with milling behavior. However, the use of 2 *N* hydrochloric acid caused difficulty in the preparation of the extracts for chromatography, because it was necessary to remove most of the ions before chromatographic separations could be made.

[2] Mention of trade-names does not imply endorsement by the U. S. Department of Agriculture.

The amount of hydrochloric acid to be removed was decreased by lowering the ratio of acid to wheat to 5:1 from the 100:1 used in the previous work. This change decreased the amount of pentosans extracted slightly but quite consistently—between 0.2 and 0.4% for the ten samples grown at Pullman, as determined by the orcinol procedure. Trials were made with 2 *N* sulfuric acid as extractant, in the hope that barium carbonate could be used for neutralization and removal of sulfate. However, the sulfuric acid extracted only one-half as much pentosan as the hydrochloric.

Wheat and bran samples were ground to pass a 40-mesh screen. Twenty-gram samples of wheat, or 10-g. samples of bran, were extracted four times with 100 ml. of hot 80% ethanol to remove extractable carbohydrates (6, 15) that might interfere with the pentose analyses. Residual alcohol was removed in the vacuum oven below 50°C. The dry sample was extracted with 100 ml. of 2 *N* hydrochloric acid at 25°C. for 6 hours with constant stirring. The acid extract was then separated by centrifuging and decanting. The volume of the supernatant extract (usually 75–80 ml.) was measured and the extract immediately heated in a boiling-water bath for 30 minutes to complete hydrolysis of the pentosans. (Water extracts were concentrated; hydrochloric acid was added to 2 *N;* and the extracts were then treated in the same manner as the acid extract.) When cool, the extract was made back to its volume before heating. Solid dry lead carbonate was then added slowly with constant stirring and with care to avoid an excess, until the extract was almost neutral. The lead-treated extract was then cooled (refrigerator, overnight) and centrifuged. Residual lead was removed from a known volume of extract by treatment with hydrogen sulfide overnight. The lead sulfide was filtered off, the paper and lead sulfide washed thoroughly, and the hydrogen sulfide removed from the filtrate and washings under reduced pressure, at the same time that the extract was concentrated to about one-half its original volume. The extract was then put through columns of ion-exchange resins, according to the procedures of Williams, Bevenue, and Washauer (16). Twenty grams of Amberlite IR-120 H and 25 g. of Duolite A-4[2] were used as cation and anion exchangers, respectively. The resins were washed thoroughly, and the treated extract and washings concentrated on the steam bath under a gentle blast of air to a volume of 5.0 ml., to give a sugar concentration of approximately 5%. The extracts then were preserved in the cold and in the presence of thymol until chromatographic separations were made.

The possibility of loss of sugars during application of the procedures for removal of hydrochloric acid was checked by comparison

of the total reducing sugar content of the extracts after the 30-minute hydrolyzing period and after the final de-ionizing and concentration. No loss of sugars occurred. However, attempts to use the ion-exchange resins immediately after the lead carbonate treatment without going through the hydrogen sulfide treatment failed, since at least one-half of the reducing sugars were lost.

Paper Chromatography: The paper-chromatographic techniques employed were essentially those of Partridge (9) and Jermyn and Isherwood (5), with modifications described by Williams and Bevenue (15). All of the chromatograms were made on Whatman No. 1 paper. The filter paper was cut into strips 18 × 46 cm. and laid out so that a center strip could be cut out for quantitative separations of the pentoses and side strips used to locate the positions of known sugars and those present in the wheat extracts. The solvent mixture used for irrigating the chromatograms was ethyl acetate:pyridine:water (8:2:1). Irrigation was continued for about 20 hours, at about 25°C., with descending solvent movement. This gave excellent separation of the xylose and arabinose. The chromatograms were washed thoroughly with anhydrous ether after removal from the tanks, and allowed to dry for about 1 hour. The positions of the sugars on the chromatograms were located by spraying with 3,5-dinitrosalicylic acid (0.5%) in 5% sodium hydroxide, and drying at 105°C. for about 4 minutes in a forced-draft oven.

Quantitative Determination of Pentoses: The wheat extracts were spotted on the filter paper by use of a micropipet which delivered 3 microliters. After the location of the pentose sugars had been made by detaching and spraying marginal guide strips, that portion of the middle strip (unsprayed) containing the sugar was cut out and the sugar eluted with distilled water by the method described by Dent (2). The eluate was made to a definite volume (usually 5 ml.), and the amount of pentose was determined by the micro-copper method of Somogyi (14) as adapted by Nelson (8). Light transmissions were measured on a Coleman Spectrophotometer (Model 11) at a wave length of 660 mμ.

The accuracy of the analytical procedures was determined by running standard solutions of xylose and arabinose through the paper-chromatographic separation and copper-reduction steps. Known solutions of xylose and arabinose (50 μg./μl.) were chromatographed and assayed, with an average error of about ±5%.

Results and Discussion

Results of pentose analyses on acid extracts of the principal series

TABLE I

MILLING EVALUATIONS OF SOME PACIFIC NORTHWEST SOFT WHEATS (1950 CROP), AND XYLOSE AND ARABINOSE CONTENTS OF ACID EXTRACTS[1] OF THE WHEATS

Station	Variety	Milling Score[2]	Straight Flour Yield[2]	Xylose[3]	Arabinose[3]
			%	%	%
Lind	Elgin	86.4	75.2	1.32	1.11
	Elmar	85.8	75.0	1.16	1.09
	Idaed	84.9	72.9	1.53	1.21
	Triplet	83.3	70.3	1.87	1.13
	Federation	81.8	71.5	1.92	1.15
	Golden	80.2	71.5	1.68	1.23
	Baart	77.4	69.3	1.90	1.25
	Brevor	77.5	71.8	1.66	1.49
	Marfed	79.5	70.0	1.80	1.38
	Rex	73.2	70.0	1.90	1.40
Pendleton	Elgin	87.8	74.7	1.01	1.53
	Elmar	89.0	75.8	1.17	1.25
	Idaed	85.0	71.1	1.20	1.30
	Triplet	83.0	69.1	1.28	1.73
	Federation	81.0	71.9	1.45	1.39
	Golden	78.9	69.2	1.51	1.58
	Baart	80.0	68.3	1.58	1.61
	Brevor	77.8	70.0	1.63	1.61
	Marfed	79.0	71.5	1.65	1.55
	Rex	73.7	67.4	1.81	1.90
Pullman	Elgin	94.2	77.9	0.95	1.60
	Elmar	89.4	75.8	1.23	1.76
	Idaed	89.2	72.2	1.28	1.50
	Triplet	86.2	71.2	1.41	1.76
	Federation	83.4	72.8	1.42	1.67
	Golden	83.0	71.1	1.43	1.43
	Baart	78.7	70.7	1.40	1.59
	Brevor	82.5	71.7	1.83	1.42
	Marfed	80.3	69.9	1.48	1.62
	Rex	76.4	69.2	1.65	1.48

[1] 2.0 N hydrochloric acid, 6 hours, 25°C.
[2] According to (12) and (13).
[3] Expressed as percent of wheat (uncorrected for moisture).

of wheats are given in Table I. Three sets of samples, each made up of ten varieties grown at a particular location, were included in the series. The ten varieties are all commercially grown, but represent an appreciable range in milling acceptability (13). In all three sets, a considerable range in xylose contents of the acid extracts was found, from near 1.0% as a minimum to around 1.8 or 1.9% as a maximum. The arabinose contents within sets varied over somewhat smaller ranges, although the over-all range for the complete series (0.81%) approaches that found with xylose (0.97%).

Correlation coefficients were calculated within sets, as well as for

the entire series; they are presented in Table II. In every case involving xylose, a significant relationship with the milling measurement was indicated; in those cases involving arabinose, significant relationships were indicated only with three combinations. It can be concluded

TABLE II

CORRELATION COEFFICIENTS FOR PENTOSE AND MILLING BEHAVIOR RELATIONSHIPS

Relationship	Samples from			All Samples
	Lind	Pendleton	Pullman	
Xylose × Milling score	−0.70*	−0.95**	−0.78**	−0.78**
Xylose × Flour yield	−0.94**	−0.73*	−0.77**	−0.68**
Arabinose × Milling score	−0.81**	−0.73*	+0.33	−0.12
Arabinose × Flour yield	−0.52	−0.75*	+0.36	−0.25

that the xylose contents of these acid extracts were related more consistently to the measures of milling acceptability used than were their arabinose contents.

The ratios of arabinose to xylose found in the acid extracts (Table I) varied from 2.4:4.0 to 6.8:4.0. Such differences might be expected to alter considerably the physical properties of pentosans made up of linear xylan chains with arabinose side chains. However, if a portion of the arabinose residues were attached to and did modify the properties of a linear xylan occurring in the wheats, the effects were not large enough to destroy the correlations with xylose contents of the acid extracts. Results presented in a following section suggest that a considerable part of the variation in ratio may arise from extraction of arabinose with little accompanying xylose from the branny parts of the wheat kernels.

A practical objective of these investigations is to develop methods for selecting the good-milling wheats from large numbers of samples. In order to be useful such methods must be both reliable and easily applicable. Determination of either pentose alone is more complicated than determination of total pentose in acid extracts; consequently determinations of xylose alone would need to give results related more closely to milling properties than determinations of total pentose, to compensate for the additional manipulations required. However, total pentose (xylose plus arabinose) in the acid extracts of wheats as given in Table I appears approximately as closely related to milling properties as the xylose alone, by comparison of the following correlation coefficients with those given in Table II:

	Lind	*Pendleton*	*Pullman*	*All samples*
Total pentose × Milling score	−0.85**	−0.95**	−0.68*	−0.74**
Total pentose × Flour yield	−0.92**	−0.83**	−0.65*	−0.75**

There appears to be no advantage gained by the separation of xylose, as compared to determination of total pentose, to justify the additional manipulations required.

Water-Soluble Pentosans; Extracts of Bran. Elder *et al.* (3) found no apparent relationship between the amounts of pentosan extracted by water from whole wheats and milling acceptability of the wheats, in contrast to the highly significant correlations found with acid extracts. Also, on the basis of examinations of milling fractions, endosperm rather than bran was considered to be the principal source of those pentosans in whole wheats responsible for the correlations found. In view of those observations and the results presented above, indicating xylose to be more closely related to milling behavior than arabinose, comparisons of the xylose and arabinose contents of acid and water extracts of whole wheats and of the acid extracts of bran were of interest. Such comparisons were made on four wheats differing in milling behavior and on the corresponding brans, with the results shown in Table III.

TABLE III

XYLOSE AND ARABINOSE IN WATER AND ACID EXTRACTS[1] OF WHOLE WHEATS AND IN ACID EXTRACTS OF BRAN PREPARATIONS
(1950 Crop Wheats, Pullman)

Wheat	Wheat				Bran	
	Water Extract		Acid Extract		Acid Extract	
Variety[2]	Xylose	Arabinose	Xylose	Arabinose	Xylose	Arabinose
	%	%	%	%	%	%
Elgin	0.29	0.31	0.95	1.60	0.90	4.58
Golden	0.34	0.30	1.43	1.43	0.79	3.95
Brevor	0.31	0.33	1.83	1.42	1.03	4.50
Rex	0.45	0.36	1.65	1.48	1.06	3.60

[1] Extraction with water or 2 N hydrochloric acid for 6 hours at 25°C.; xylose and arabinose in extracts expressed as percent of wheat or bran (uncorrected for moisture).
[2] Milling scores (13): Elgin, 94.2; Golden, 83.0; Brevor, 82.5; Rex, 76.4.

The water-extracted pentosans from these four wheats are less variable in the proportions of xylose and arabinose they contain than are the acid-extracted pentosans. In the latter, the xylose contents vary more than the arabinose contents.

The amounts of xylose extracted from the bran preparations are so small that bran can be expected to contribute little to the xylose in the acid extracts of whole wheats. The preparations used here were low in endosperm content, although they probably contained proportionately more of the outer bran coats than of the inner coats, as compared to wheat. However, if the bran preparations are considered to be representative of the total bran, and the total bran is estimated to amount to one-seventh of the kernel by weight, the maximum amount of xylose derived from bran in the whole wheat extracts is 0.15%.

Three to five times more arabinose than xylose was extracted from these bran preparations. No information on samples from stations other than Pullman were obtained; but further work on bran extracts did not appear worth while in view of the small fraction—less than one-sixth—of total pentosan extracted. The Elgin and Rex preparations were found to contain 36% total pentosans (1), and yielded only 5.5 and 4.5%, respectively, on acid extraction. Loska and Shellenberger (7) found 30% total pentosans in similar branny material.

These values with bran and bran extracts are in marked contrast to those with whole wheat. The total pentosan contents of the Rex and Elgin wheats were determined (1); 7% and 6.36%, respectively, were found. The pentosans present in the 2 *N* hydrochloric acid extracts thus represent 45% and 40% of the total whole wheat pentosans.

The observations on these four wheats and the bran preparations from them are consistent with the earlier conclusion regarding the relationships of pentosans in these Pacific Northwest soft wheats to their milling properties; that is, that endosperm rather than bran pentosans are responsible for the correlations observed. The finding that the pentose extracted from bran is very largely arabinose was unexpected, but it is in agreement with the correlations observed between xylose and milling properties, as well as with the predominant role of endosperm pentosans in those correlations.

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Literature Cited

1. Association of Official Agricultural Chemists. Official methods of analysis (7th ed.), Assoc. Offic. Agr. Chemists: Washington, D. C. (1950).

2. Dent, C. E. The amino-aciduria in Fanconi syndrome. A study making extensive use of techniques based on paper partition chromatography. Biochem. J. **41:** 240–253 (1947).
3. Elder, Angeline H., Lubisich, T. M., and Mecham, D. K. Studies on the relation of the pentosans extracted by mild acid treatments to milling properties of Pacific Northwest wheat varieties. Cereal Chem. **30:** 103–114 (1953).
4. Hoffpauir, C. L. Report on starch in plants. The use of the anthrone reaction. J. Assoc. Offic. Agr. Chemists **35:** 398–401 (1952).
5. Jermyn, M. A., and Isherwood, F. A. Improved separation of sugars on a paper partition chromatogram. Biochem. J. **44:** 402–407 (1949).
6. Koch, R. B., Geddes, W. F., and Smith, F. The carbohydrates of *Gramineae*. I. The sugars of the flour of wheat (*Triticum vulgare*). Cereal Chem. **28:** 424–430 (1951).
7. Loska, S. J., Jr., and Shellenberger, J. A. Determination of the pentosans of wheat and flour and their relation to mineral matter. Cereal Chem. **26:** 129–139 (1949).
8. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153:** 375–380 (1944).
9. Partridge, S. M. Application of the paper partition chromatogram to the qualitative analysis of reducing sugars. Nature **158:** 270–271 (1946).
10. Perlin, A. S. Isolation and composition of the soluble pentosans of wheat flours. Cereal Chem. **28:** 370–381 (1951).
11. Perlin, A. S. Structure of the soluble pentosans of wheat flour. Cereal Chem. **28:** 382–393 (1951).
12. Seeborg, E. F. A modified flow sheet for Buhler mills. Milling Production **15:** No. 1 (1950).
13. Seeborg, E. F. Evaluation of wheat milling characteristics by laboratory methods. Trans. Am. Assoc. Cereal Chem. **11:** 1–5 (1953).
14. Somogyi, M. A new reagent for the determination of sugars. J. Biol. Chem. **160:** 61–68 (1945).
15. Williams, K. T., and Bevenue, A. The chromatographic examination of sugars in wheat flour. Cereal Chem. **28:** 416–423 (1951).
16. Williams, K. T., Bevenue, A., and Washauer, Barbara. A study of the use of ion-exchange resins for the removal of non-sugar reducing substances in the analysis of fresh and dehydrated vegetables for reducing sugars. J. Assoc. Offic. Agr. Chemists **33:** 986–994 (1950).

ON THE CHANGES IN THE SOLUBLE SOLIDS AND CARBOHYDRATES OF BREAD CRUMB DURING THE AGING OF CONVENTIONAL AND BACTERIAL ALPHA-AMYLASE-SUPPLEMENTED WHITE BREADS[1]

S. S. Jackel, W. E. Schaeder, and A. S. Schultz[2]

ABSTRACT

The soluble-polysaccharide content of conventional and bacterial alpha-amylase-supplemented white bread was determined during aging at various temperatures, by measurement of the increase in reducing sugars resulting from acid hydrolysis of the aqueous extracts of the crumbs. Approximately 4% of the crumb polysaccharides in conventional bread were soluble in water at 25°C. when the bread was oven-fresh. Storage at 8°, 30°, and 50°C. served to decrease the amounts of soluble-polysaccharide material by values of 33%, 16%, and 7%, respectively. The decreases in soluble polysaccharides were complete in each case within the first 17 hours of storage. In breads supplemented with bacterial alpha-amylase there were significant increases in the amounts of soluble polysaccharides during storage at 30° and 50°C. but not during storage at 8°C. These breads contained twice as much soluble-polysaccharide material as their conventional counterparts when oven-fresh and four to five times as much after storage at 30° or 50°C. The soluble reducing sugar content of the oven-fresh enzyme-treated breads was essentially the same as that of the conventional breads and remained constant during storage despite the increase in soluble polysaccharides. It was concluded, in agreement with and in extension of previous reports, that the bacterial alpha-amylase functioned both during baking and during subsequent storage to degrade the starch to moieties small enough to be soluble but not small enough to be reducing.

Authoritative reviews concerned with the problem of bread staling, such as that of Geddes and Bice (10), generally mention a decrease in the amount of soluble crumb starch as one of certain characteristic changes which take place during the normal aging of bread. Although the literature is well documented in this respect with reports from various laboratories (4, 6, 8, 14, 16, 17, 22), a number of papers have questioned whether changes in soluble starch do occur on staling and have criticized Katz' classical method (15) for determining soluble starch in bread.

Thus, Fisher (8) stated that it was impossible to express the decrease in soluble starch with any degree of accuracy, and Fuller (9) expressed the opinion that the time involved in determining soluble polysaccharides was not warranted by the results. Karácsonyi (13) re-

[1] Manuscript received April 27, 1953.
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ported that the change in water-soluble polysaccharides during staling was at the most very slight; Esselbaugh (7) found a progressive increase in the total solids of the water extract over a 72-hour period; and Bechtel and Meisner (5) recently concluded that changes in soluble starch could not be related to the staling process. Most recently, in a communication from this laboratory (11), evidence was presented indicating that starch staling, as evaluated by decreased susceptibility to beta-amylase (12), is confined primarily to the insoluble part of the crumb.

It was the purpose of the present investigation to obtain additional information concerning the nature of the changes in the soluble extract during staling and to determine whether differences in these respects exist between conventional white breads and those supplemented with bacterial alpha-amylase. It was anticipated that such data, concerned with the influence of time and temperature of storage on the amounts of water-extractable solid matter, reducing sugars, and soluble polysaccharides, would be of value in evaluating the question of relationship between soluble extract and the staling phenomenon.

Materials and Methods

Sample Preparation. Conventional and bacterial alpha-amylase-supplemented white breads (12, 19) were prepared on a commercial scale in 50-lb.-flour batches by the sponge dough process and sealed in moisture-proof cellophane bags after reaching room temperature. All samples were stored at the specified temperatures for future testing. Water-soluble extracts of the breads were prepared immediately before analysis by extracting 40 g. of interior bread crumb twice, with 100-ml. portions of water, and diluting the filtered extracts to 200 ml. Details of the extraction procedure have been given previously and it was established that the efficiency of extraction is in the range of 69 to 85% (11).

Analytical Procedures. All results were calculated and expressed on a basis representing 20 g. of crumb. *Water-soluble solids* were determined by drying a 10-ml. aliquot of the soluble extract (in a tared 55-mm.-diameter aluminum drying dish provided with a press-in cover) to dryness on a steam bath and then to constant weight in a forced-draft air oven maintained at 130°C. (1). *Water-soluble reducing sugars before acid hydrolysis* were determined by defecating a 30-ml. aliquot of the soluble extract with 5 ml. of a saturated solution of neutral lead acetate, diluting to 100 ml., filtering through folded Whatman No. 12 filter paper, deleading with anhydrous so-

dium oxalate, filtering again, and analyzing the clear filtrate by the Lane-Eynon titration procedure (3).

Water-soluble reducing sugars after acid hydrolysis were measured by transferring a 30-ml. aliquot of the soluble extract to a 250-ml. volumetric flask, adding 70 ml. of water and 10 ml. of 7.8 *N* hydrochloric acid (sp. gr. 1.125), attaching a reflux condenser, and heating for 2.5 hours in a boiling water bath, neutralizing with anhydrous sodium carbonate after cooling, defecating by adding 20–40 ml. of a saturated solution of neutral lead acetate, diluting to 250 ml., and continuing with filtration, deleading, filtration, and analysis as directed for *reducing sugars before acid hydrolysis. Water-soluble polysaccharides* were calculated as dextrose by difference between *reducing sugars before and after acid hydrolysis* and as starch by multiplying the difference by 0.9.

Results and Discussion

Conventional White Bread. Most of the soluble-polysaccharide values reported in the literature are expressed in terms of soluble starch and were obtained by procedures—following either Katz' original technique (15) or subsequent improvements such as that of Schoch and French (17)—involving gravimetric determination of the amount of solids precipitated by excess alcohol from an aqueous extract of the bread crumb. In view of the controversial nature of the various reports it appeared advisable to determine soluble polysaccharides by means of an alternative technique based on determination of the amounts of reducing sugars, before and after acid hydrolysis, in an aqueous extract of the bread crumb. This method is recommended by the Association of Official Agricultural Chemists (2) for use in the determination of soluble starch in grains and it would appear to be applicable to the measurement of soluble polysaccharides (the sum of soluble starch, pentosans, starch-degradation products, etc.) in bread.

Results obtained by this procedure are given in Table I along with values for solid matter and reducing sugars. All data are expressed as quantities present in the soluble extract from 20 g. of crumb. Although the data show some variation with time and temperature of storage, the most striking observation is that only relatively small proportions of the crumb polysaccharides and crumb solid matter appeared in the water extracts. Thus, it may be calculated that 3–4% of the crumb polysaccharides and 11–14% of the crumb solids were represented by the material in the aqueous extract. By contrast, very much larger proportions of the reducing sugars were extracted, cal-

TABLE I

INFLUENCE OF TIME AND TEMPERATURE OF STORAGE ON SOLIDS AND CARBOHYDRATES IN THE SOLUBLE EXTRACT OF CONVENTIONAL WHITE BREAD[1]

Conditions of Storage		Solids	Reducing Sugars[2]		Soluble Polysaccharides[3]	
			Before Hydr.	After Hydr.	As Dextrose	As Starch
°C.	hrs.	g.	g.	g.	g.	g.
8	0	1.36	0.44	0.82	0.38	0.34
	17	1.62	0.61	0.90	0.29	0.26
	41	1.63	0.54	0.88	0.34	0.31
	65	1.62	0.59	0.87	0.28	0.25
	89	1.59	0.51	0.81	0.30	0.27
30	0	1.36	0.44	0.82	0.38	0.34
	17	1.35	0.50	0.81	0.31	0.28
	41	1.49	0.51	0.86	0.35	0.31
	65	1.65	0.52	0.93	0.41	0.37
	89	1.56	0.54	0.91	0.37	0.33
50	0	1.36	0.44	0.82	0.38	0.34
	17	1.31	0.48	0.80	0.32	0.29
	41	1.28	0.42	0.79	0.37	0.33
	65	1.49	0.43	0.82	0.39	0.35
	89	1.35	0.50	0.83	0.33	0.30

[1] All data are reported as amounts in the aqueous extract from 20 g. of crumb.
[2] Expressed as dextrose before or after acid hydrolysis.
[3] Expressed as dextrose by difference between reducing sugars before and after acid hydrolysis or as starch by multiplying the difference by 0.9.

culation indicating recovery of 63–87% of the reducing sugars present in the crumb. (In arriving at these estimates conventional values (20) for bread of about 40% moisture, 52% total carbohydrates as dextrose, 3.5% reducing sugars as dextrose, and 42.5% starch were used. The conventional values checked well with analytical determinations on the breads used in these studies.)

Evidence that the extraction procedure employed was reasonably quantitative (69 to 85%) was presented previously (11) in connection with the studies on the susceptibility of the water extracts to hydrolysis by beta-amylase. A recheck of the conditions of extraction by measurement of solids, reducing sugars, and soluble polysaccharides in the aqueous extracts provided further confirmation that the procedure was satisfactory, identical results being obtained regardless of the length of extraction (2 minutes to 4 hours) or the volume of extractant (100 or 200 ml. of water per 40 g. of crumb).

Evidence concerning the analytical procedure employed was reviewed critically (2) by the Association of Official Agricultural Chemists and led to designation as an official method for soluble starch in grains. Although this procedure cannot be considered specific for

soluble starch when applied to bread (because of the presence of soluble pentosans and various starch-degradation products hydrolyzable by acid to reducing substances), it appears to afford a thoroughly reliable composite measure of the soluble polysaccharides. The actual data indicate that soluble starch is the major component in bread extracts, since the values obtained for soluble polysaccharides (3–4% of the crumb starch) are of the same order of magnitude as those obtained for soluble starch in other laboratories by specific analytical procedures. Calculation from published data (5, 17) indicates values for soluble starch in the range of 3–9% of the crumb starch with oven-fresh bread and 2–4% with stale bread. These data are all in good agreement with previous data from this laboratory (11) indicating that less than 10% of the crumb starch susceptible to attack by beta-amylase is water-soluble.

The data of Table I indicate that the amounts of the various soluble components in the aqueous extracts were influenced by the time and temperature of storage, but that the pattern of change was considerably different from that ordinarily followed by the intact bread. Thus, the amounts of soluble polysaccharides decreased by approximately 25% during storage at 8°C., but the decrease was complete within the first 17 hours and did not change on further storage. During storage at 30° and 50°C. there was little change, probably within the limits of experimental error, during the 89-hour storage period. In contrast, the amounts of solid matter in the water extracts increased by about 20% during storage at 8° and 30°C. At 50°C. there was essentially no change in the amount of soluble solids during the storage period.

The increase in the amount of solid matter extracted from the crumbs of breads aged at 8° and 30°C. was not unexpected in view of Esselbaugh's report (7) indicating a progressive increase in the total solids of water extracts during storage for 72 hours at 30°C. The significance of the increase is not understood, but it probably is related to the influence of the storage conditions on the colloidal properties of the bread with consequent effect on the efficiency of extraction. In a previous communication (11) it was demonstrated that extraction efficiencies (with respect to fermentable carbohydrates) were 69% when breads were oven-fresh or aged at 50°C., 75% when stored at 30°C., and 85% when aged at 8°C. It seems reasonable, in view of the correlation between improvement in efficiency of extraction and maintenance of conditions known to promote staling, to speculate that the physical nature of fresh bread prevents efficient extraction and that the observed increases in soluble solids during staling reflect

improvements in extractability of the bread crumbs.

It appeared probable, in view of the greater extractability of stale bread, that proper interpretation of the soluble-polysaccharide data of Table I may have been complicated by the opposing influences of decreased soluble polysaccharide content and increased extraction. Recalculation of these data to an identical soluble-solids basis provides a method for eliminating the effect of differences in efficiency of extraction. The results of such calculations are shown in Table II. It

TABLE II

INFLUENCE OF TIME AND TEMPERATURE OF STORAGE ON THE POLYSACCHARIDE CONTENT OF THE SOLUBLE CRUMB SOLIDS OF CONVENTIONAL WHITE BREAD[1]

Conditions of Storage		Composition of Soluble Solids		
		Reducing Sugars[2]		Polysaccharides[3]
		Before Hydr.	After Hydr.	
°C.	hrs.	%	%	%
8	0	32.4	60.3	25.2
	17	37.6	55.6	16.1
	41	33.1	54.0	18.8
	65	36.4	53.7	15.6
	89	32.1	50.9	17.0
30	0	32.4	60.3	25.2
	17	37.0	60.0	20.7
	41	34.2	57.7	21.1
	65	31.5	56.4	22.2
	89	34.6	58.3	21.3
50	0	32.4	60.3	25.2
	17	36.6	61.1	21.9
	41	32.8	61.7	26.0
	65	28.8	55.0	23.5
	89	37.0	61.5	22.0

[1,2,3] Calculated from the data of Table I. Refer to Table I for appropriate footnotes. Polysaccharides expressed as starch.

is likely that the recalculated data provide a more reasonable basis from which to evaluate the influence of storage conditions on changes in the soluble polysaccharides.

The known influence of temperature on bread staling (4, 6, 9, 12, 18) is seen to be reproduced, at least qualitatively, in the soluble-polysaccharide data of Table II. Thus, 25.2% of the soluble solids was polysaccharide in nature when the breads were oven-fresh and this value decreased to 16.9, 21.3, and 23.4% (average of 17- to 89-hour data) after storage of the breads at 8°, 30°, and 50°C., respectively. It is most noteworthy, however, that the changes in soluble polysac-

charides were complete in each case within the first 17 hours of storage. Inasmuch as bread undergoes only a portion of its staling during the first 17 hours, it appears likely that changes in the polysaccharide composition of the soluble solids are complete at a time when bread is still considered fresh by consumer judgment.

Bacterial Alpha-Amylase-Supplemented Bread. Previous reports from this and other laboratories (19, 21) indicated that bread baked with low levels of bacterial alpha-amylase (in the range of 3–6 Sandstedt, Kneen, and Blish units per pound of bread) remained fresh for long periods of time. It was demonstrated (19) that the crumb starch in these breads did not follow the same pattern of change characteristic of conventional breads, susceptibility to beta-amylase remaining at high values during prolonged storage. Furthermore, the amounts of *attackable* starch soluble in water increased appreciably during storage, the data (11) indicating that the enzyme continued to function after baking to degrade the *attackable* insoluble starch to soluble moieties.

The data of Table III present evidence confirming and extending the previous observations with respect to the unique pattern of change in the soluble components of bread baked with bacterial alpha-

TABLE III

INFLUENCE OF TIME AND TEMPERATURE OF STORAGE ON SOLIDS AND CARBOHYDRATES IN THE SOLUBLE EXTRACT OF WHITE BREAD BAKED WITH SIX SKB UNITS OF BACTERIAL ALPHA-AMYLASE[1]

Conditions of Storage		Solids	Reducing Sugars[2]		Soluble Polysaccharides[2]	
			Before Hydr.	After Hydr.	As Dextrose	As Starch
°C.	hrs.	g.	g.	g.	g.	g.
8	0	1.60	0.40	1.14	0.74	0.67
	17	2.14	0.67	1.45	0.78	0.70
	41	2.03	0.62	1.24	0.62	0.56
	65	1.95	0.63	1.27	0.64	0.58
	89	1.92	0.60	1.20	0.60	0.54
30	0	1.60	0.40	1.14	0.74	0.67
	17	2.27	0.55	1.74	1.19	1.07
	41	1.96	0.37	1.55	1.18	1.06
	65	2.29	0.50	1.83	1.33	1.20
	89	3.08	0.61	2.45	1.34	1.66
50	0	1.60	0.40	1.14	0.74	0.67
	17	3.23	0.56	2.71	2.15	1.93
	41	2.60	0.44	2.08	1.64	1.48
	65	2.53	0.45	2.05	1.60	1.44
	89	3.12	0.64	2.52	1.88	1.69

[1,2] Refer to Table I for the appropriate footnotes.

amylase. At both 30° and 50°C., temperatures at which the residual alpha-amylase might be expected to continue functioning at significant rates, the amounts of solid matter extracted from 20 g. of crumb increased from 1.6 g. when the bread was oven-fresh to final values (after 89 hours of storage) of 3.1 g.

It is evident from the data that the solid matter solubilized during storage was primarily carbohydrate in nature. Thus, of the 1.5-g. increase in soluble solids, 1.0 g. (approximately 70%) was in the soluble-polysaccharide fraction and 0.2 g. (about 15%) was in the reducing sugar (before acid hydrolysis) component. It appears probable that the increase in extractable solids was a consequence of amylolytic activity and hence was confined to the carbohydrate portion of the crumb. At 8°C., a temperature at which the rate of enzyme action would be expected to be low, the behavior of the soluble fraction resembled that of conventional bread. Thus, there was evidence of a decrease in soluble polysaccharides during the storage period. These data suggest that it is the continued action of the residual alpha-

TABLE IV

COMPARISON OF CHANGES IN THE SOLUBLE CRUMB COMPONENTS OF CONVENTIONAL AND BACTERIAL ALPHA-AMYLASE-SUPPLEMENTED WHITE BREADS[1]

		Fresh Breads[2]	Stored Breads[3]		
			8°C.	30°C.	50°C.
		g.	g.	g.	g.
Solids	Conv.[4]	1.36	1.62	1.51	1.36
	6 SKB[5]	1.60	2.01	2.40	2.87
	Change	+0.24	+0.39	+0.89	+1.51
Polysaccharides[6]	Conv.	0.34	0.27	0.32	0.32
	6 SKB	0.67	0.59	1.25	1.64
	Change	+0.33	+0.32	+0.93	+1.32
Reducing sugars[7] (before)	Conv.	0.44	0.56	0.52	0.46
	6 SKB	0.40	0.63	0.51	0.52
	Change	−0.04	+0.07	−0.01	+0.06
Reducing sugars[8] (after)	Conv.	0.82	0.86	0.88	0.81
	6 SKB	1.14	1.29	1.89	2.34
	Change	+0.32	+0.43	+1.01	+1.53

[1] Calculated from data of Tables I and III. All values represent amounts in the aqueous extract from 20 g. of crumb.
[2] Fresh bread analyzed within 2 hours after baking.
[3] The average of data obtained at 17, 41, 65, and 89 hours after baking.
[4] Conventional sliced white bread.
[5] White bread baked with the inclusion of six Sandstedt, Kneen, and Blish units of bacterial alpha-amylase per pound of bread.
[6] Calculated by multiplying the difference between reducing sugars, before and after acid hydrolysis, by the factor 0.9 to express results as starch.
[7] Reducing sugars before acid hydrolysis.
[8] Reducing sugars after acid hydrolysis.

amylase during the storage period which is largely responsible for the difference between enzyme-treated and conventional breads.

To facilitate comparison of conventional and bacterial alpha-amylase breads the data of Tables I and III are summarized and combined in Table IV. Examination of the analytical values indicates that the amounts of extractable reducing sugars (before acid hydrolysis) were essentially the same for both the enzyme breads and their conventional counterparts. Differences between the two types of bread are evident, however, in the data concerned with soluble solids and soluble polysaccharides. As discussed previously, the increases in soluble solids appear to result mainly, if not entirely, from the increases in soluble-polysaccharide material.

It is of particular interest to note that the alpha-amylase breads contained approximately twice as much soluble-polysaccharide material as the controls when oven-fresh, that this value remained essentially unchanged after storage at 8°C., and that the ratio increased to fourfold after storage at 30°C. and to fivefold after storage at 50°C. The most likely explanation for this phenomenon is that the enzyme functioned to degrade the insoluble starch to soluble forms both during baking and during the subsequent storage periods. The lack of increase with the breads stored in the refrigerator probably implies that the enzyme activity was appreciably retarded by the reduced temperature. The lack of increase in the amounts of reducing sugars (before acid hydrolysis) at any storage temperature suggests that the soluble moieties were not degraded sufficiently to be reducing without further hydrolysis.

Acknowledgment

The authors wish to thank Mr. F. D. Schoonover for baking the breads used in these studies and Mr. W. E. Maynard for carrying out the analytical determinations.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.), Am. Assoc. Cereal Chemists: St. Paul, Minn. (1947), p. 23.
2. Association of Official Agricultural Chemists. Official methods of analysis (7th ed.), Assoc. Offic. Agr. Chemists: Washington, D.C. (1950), p. 348.
3. Association of Official Agricultural Chemists. Official methods of analysis (7th ed.), Assoc. Offic. Agr. Chemists: Washington, D.C. (1950), p. 506.
4. Bachrach, H. L., and Briggs, D. R. Studies on bread staling. II. Water relationships during staling of bread crumb and the retrogradation of starch. Cereal Chem. **24:** 492–506 (1947).
5. Bechtel, W. G., and Meisner, D. F. Present status of the theory of bread staling. Food Technology **5:** 503–505 (1951).
6. Bice, C. W., and Geddes, W. F. Studies on bread staling. IV. Evaluation of methods for the measurement of changes which occur during bread staling. Cereal Chem. **26:** 440–465 (1949).
7. Esselbaugh, N. C. Some factors involved in the staling of whole wheat bread. Ohio State Univ., Abs. Doctoral Dissertation, No. 44: 165–171 (1944); Exp. Sta. Rec. **93:** 219 (1945).

8. FISHER, E. A. The staling of bread: A review of existing knowledge of the problem. Research Assoc. of Brit. Flour Millers. Spec. Report No. 11 (1934).
9. FULLER, C. H. F. Starch and bread-staling. J. Soc. Chem. Ind. **57:** 562–568 (1938).
10. GEDDES, W. F., and BICE, C. W. The role of starch in bread staling. Q.M.C. 17–10. War Department. Office of the Quartermaster General, Washington, D.C. (1946).
11. JACKEL, S. S., SCHAEDER, W. E., and SCHULTZ, A. S. Susceptibility to hydrolysis by beta-amylase of the soluble and insoluble fractions of the crumb of conventional and bacterial alpha-amylase supplemented white bread. Cereal Chem. **30:** 190–201 (1953).
12. JACKEL, S. S., SCHULTZ, A. S., SCHOONOVER, F. D., and SCHAEDER, W. E. The decrease in susceptibility of bread crumb starch to beta-amylase during staling. Cereal Chem. **29:** 190–199 (1952).
13. KARÁCSONYI, L. Die Rolle der Kohlenhydrate und Eiweisskörper beim Altbackenwerden des Brotes. Z. Untersuch. Lebensm. **56:** 479–484 (1928).
14. KATZ, J. R. The cause of the staling of bread. Chem. Weekbad. **9:** 1023–1058 (1912).
15. KATZ, J. R. Gelatinization and retrogradation of starch in relation to the problem of bread staling. *In* A comprehensive survey of starch chemistry, ed. by R. P. Walton. Vol. I. The Chem. Catalog Co.: New York (1928).
16. LINDET, L. Sur les états que présente l'amidon dans le pain tendre et dans le pain rassis. Bull. soc. chem. **27:** 634–639 (1902).
17. SCHOCH, T. J., and FRENCH, D. Studies on bread staling. I. The role of starch. Cereal Chem. **24:** 231–249 (1947).
18. SCHULTZ, A. S., and LANDIS, Q. The hydrolysis of starch in bread by flour and malt amylase. Cereal Chem. **9:** 305–310 (1932).
19. SCHULTZ, A. S., SCHOONOVER, F. D., FISHER, R. A., and JACKEL, S. S. Retardation of crumb starch staling in commercial bread by bacterial alpha-amylase. Cereal Chem. **29:** 200–211 (1952).
20. SHERMAN, H. C. Chemistry of food and nutrition (7th ed.). The Macmillan Co.: New York (1946), p. 622.
21. STONE, I. Retarding the staling of bakery products. U.S. Patent No. 2,615,810. Assigned to the Wallerstein Co., New York (1952).
22. WHYMPER, R. Conditions that govern staleness in bread. Sci. Am. Mo. **1:** 96 (1920).

THE RELATIONSHIP OF THE SEDIMENTATION OF BREAD CRUMB SUSPENSION TO AGE[1, 2]

O. J. Banasik and R. H. Harris[3]

ABSTRACT

Sedimentation rate of bread crumb suspensions may be conveniently followed by photoelectric measurements of the light scattered by the crumb particles. A suspension containing 1.0% of weight of dry crumb was the most satisfactory. Sedimentation rate decreased as bread aged, whereas inclusion of potato flour or polyoxyethylene monostearate in the baking formula increased the rate. Breads stored at different moisture contents for 24 hours gave higher sedimentation values as the moisture was lowered.

A correlation of −0.93 was obtained between crumb firmness and sedimentation rate when the influence of crumb moisture was eliminated by partial correlation methods.

Several methods are employed to measure the complex changes which occur as bread ages. These have been evaluated by Bice and Geddes (1), who state that any method can measure, at best, only a few of the factors involved in the subjective integrated assessment of staling. They found that the Farinograph consistency, swelling power, and soluble starch methods gave results which changed in a curvilinear manner with time. Crumb compressibility showed the greatest change in the initial 20-hour period. Only crumbliness values gave an approximately linear relationship with time. Evidently the percent change of staling with time as determined by these different methods cannot be expected to be highly correlated.

This present paper describes a means of measuring variations in the density of aqueous dispersions of bread crumb and another method for following some of the changes involved in bread aging. The results were compared with other procedures and analyzed as to the effects of adjunct, baking methods, and crumb age.

Materials and Methods

Commercially baked bread containing shortening was used in the standardization of the method. It was stored in sealed moisture-proof plastic film bags until used.

Several 350-g. loaves were experimentally baked to provide sufficient material to test the effect of adjunct and formula on the staling

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[2] Publication approved by the Director of the North Dakota Agricultural Experiment Station.
[3] Address: North Dakota Agricultural Experiment Station, Fargo, North Dakota.

rate. The baking methods employed were the malt-phosphate-bromate (5% sucrose) with 2-hour fermentation and lean and rich formulas with 3-hour fermentation (Harris, Sibbitt, and Banasik, 4). Cooked potato flour in 1 and 4% concentrations and polyoxyethylene monostearate (at 0.5% concentration and denoted as stearate in this report) were used as adjuncts. The loaves were sliced, double-wrapped in waxed paper, sealed, and stored in tins in the laboratory until analyzed.

Preparation of Crumb Suspension. The outer crumb and crust were removed from the bread and the inner crumb was shredded in a Waring Blendor for approximately 1 minute. Two methods of preparing the crumb suspension were investigated. In the first, 10 g. (dry basis) of the shredded crumb was rubbed through a wire sieve (81 openings per sq. cm.) with distilled water. The crumb particles were rendered finer by forcing them through a second wire sieve having 144 openings per sq. cm. with rinsing, and the final volume was made up to 500 ml., representing a 2% suspension. In the second method, 200 ml. of distilled water and 10 g. of crumb (dry basis) were macerated in the Blendor at the lowest speed obtainable for 5 minutes. The suspension was then transferred quantitatively to a graduated cylinder and made up to 500 ml. A drop or two of toluene was added as a preservative.

Measurement of Crumb Hydration. A combination of the Cathcart-Luber method (2) and the starch swelling power method of Harris and Jesperson (3) was selected to provide an accurate means of determining the hydration capacity of the bread as it aged. This procedure was as follows: after standing 0.5 to 1 hour, 50 ml. of the suspension was centrifuged for 10 minutes at 1800 r.p.m. in an International-type SB centrifuge. The centrifuge tubes were drained and carefully wiped dry. Aliquots from the central portion of the precipitate were then removed, the moisture content determined, and hydration calculated as grams of water per gram of dry material (3).

Determination of Crumb Sedimentation Rate. The apparatus employed for determining sedimentation rate is shown in Fig. 1. A light beam produced by a 100-watt incandescent bulb regulated by a voltage stabilizer is scattered at right angles to the incident beam by the suspended crumb particles. This diffracted light passes through 2.5×0.5-cm. slits centered 2.5 cm. from the liquid level and activates two photoelectric cells placed in opposite sides of the chamber and connected in parallel to a sensitive galvanometer. A variable resistance was shunted across the line, so that the current produced by the photoelectric cells could be adjusted to cause the values to fall within the scale range of the galvanometer. The suspension tubes measured

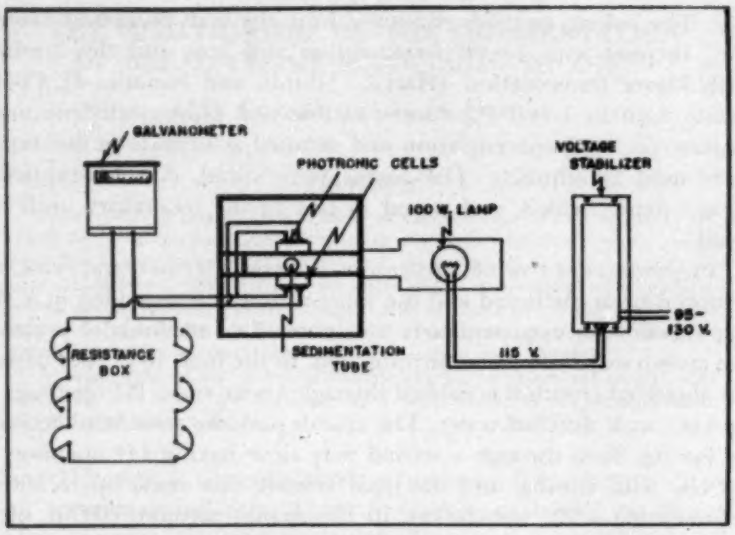

Fig. 1. Line drawing of the apparatus for determining crumb sedimentation rate by light reflection.

20 mm. × 175 mm. and were of uniform size. For determining sedimentation rates sufficient suspension was poured into the tube to produce a suitable and uniform depth of liquid, the tube and contents were thoroughly shaken and placed in the apparatus, and a stop watch was started. Galvanometer readings were taken at 1-minute intervals. The initial reading was arbitrarily assigned a relative value of 100% reflected light and subsequent readings were expressed as percentages of the first reading.

Preliminary experiments showed that the rate of fall might be related to bread age. By plotting changes in galvanometer readings *vs.* time, a logarithmic curve was obtained, which when transformed to logarithmic coordinates, yielded a straight line. Thus the data followed the power law: $y = ax^n$, or $\log y = \log a + n \log x$ where x = time, minutes, y = % light diffracted, a = intercept, and n = slope. As the crumb aged the slope (n) increased, indicating that the rate of fall of the crumb was also increasing. To simplify the determination of crumb sedimentation rates (slope), the ratio as percent of light diffracted over two portions of the curve representing 15 and 5 minutes was calculated (Fig. 2). This was denoted as the crumb sedimentation value (%). It can be shown that this value is still a function of the slope where: $n = \frac{\log y - \log y_1}{\log x - \log x_1}$. Since $\log x - \log x_1$ can be written $\log 15 - \log 5$ and is constant, then $n\ (\log 15 - \log 5) = \log y -$

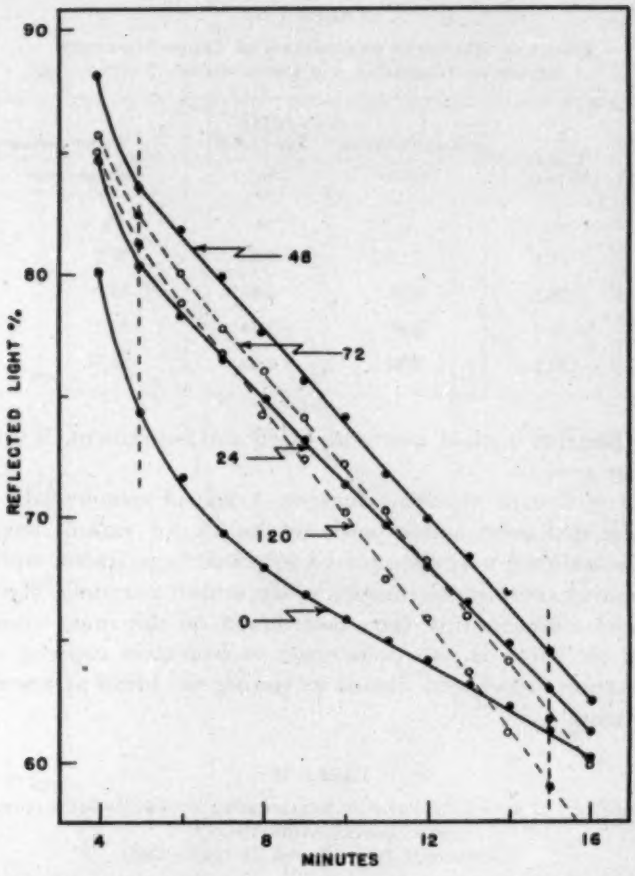

Fig. 2. Effect of bread age on the sedimentation rate of bread crumb in aqueous suspension.

log y_1 or $3^n = y/y_1$. The standard deviation between duplicate sedimentation determinations on 30 loaves of commercially baked bread 0–72 hours in age was ± 0.85. The variance for the differences between loaves was not significant at the 5% point.

Results and Discussion

Effect of Method of Preparation of the Suspension. The data in Table I show that the Blendor suspensions gave slightly lower results than the sieve method, probably because the crumb particles in the former were more finely divided and a slightly greater degree of hydration may have caused them to settle out at a faster rate. Because the

TABLE I

EFFECT OF METHOD OF PREPARATION OF CRUMB SUSPENSION ON CRUMB HYDRATION AND SEDIMENTATION VALUES

Bread Age	Crumb Moisture	Crumb Hydration $\frac{H_2O}{Dry\ Crumb}$		Crumb Sedimentation	
		Blendor	Sieve	Blendor	Sieve
Hours	%	g.	g.	%	%
0	41.3	4.18	3.99	90.7	90.9
24	39.7	2.83	2.75	85.1	88.7
48	38.6	2.63	2.61	84.9	88.7
72	38.1	2.58	2.60	84.5	88.5

Waring Blendor method was more rapid and convenient, it was used in further work.

Effect of Crumb Moisture Content. A loaf of commercially baked bread was quartered and exposed to the air for various lengths of time. The quarters were then placed in plastic bags, sealed, and stored for 24 hours to permit equalization of the moisture content. Hydration values and sedimentation rates determined on the inner crumb are recorded in Table II. Small increases in hydration capacity and in sedimentation values were caused by storing the bread at lower moisture contents.

TABLE II

EFFECT OF MOISTURE CONTENT OF BREAD CRUMB ON CRUMB HYDRATION AND SEDIMENTATION VALUES
(Commercial White Bread 24 Hours Old)

Exposure to Air	Crumb Moisture	Hydration $\frac{H_2O}{Dry\ Crumb}$	Sedimentation Value
Hours	%	g.	%
0	37.2	2.81	87.1
1	36.2	2.83	87.9
3	35.0	2.97	88.3
5	31.8	3.13	88.9

Effect of Concentration and Crumb Suspension Age. Figure 3 illustrates the effect of crumb concentration. For each concentration the resistance was adjusted until a maximum galvanometer reading was obtained. The peculiar behavior of the 2% concentration may be due

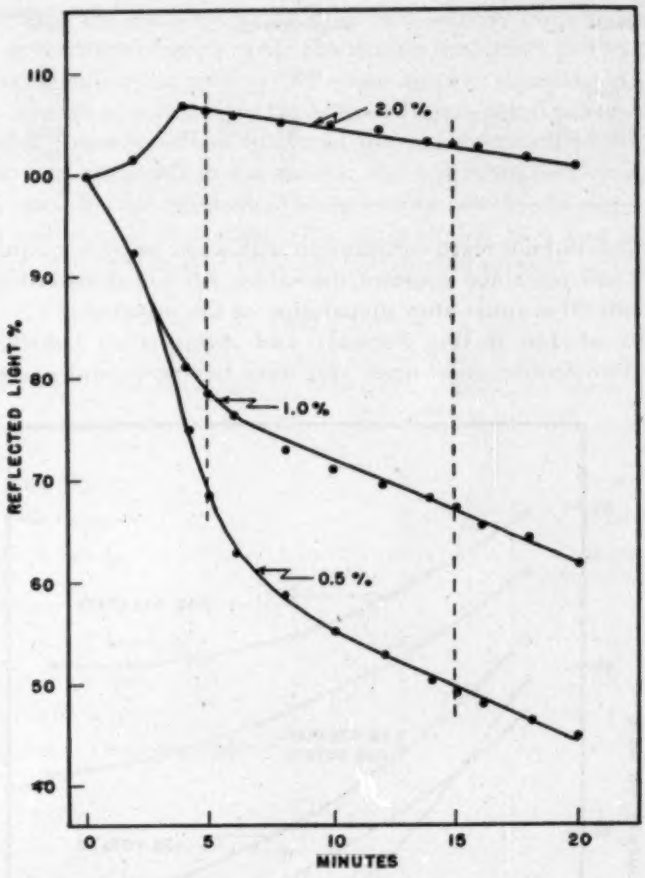

Fig. 3. Effect of crumb concentration of fresh bread on sedimentation rate.

to an initial mutual interference of crumb particles. For the 1.0 and 0.5% suspensions, the greatest change occurred in the first 5 minutes. A nearly linear relationship exists over the 5- to 20-minute period for each concentration. Comparison of the three curves over this period showed that the 2% concentration had the best replicability, but the 1% and 0.5% suspensions produced the greatest change. It was decided to employ the 1% concentration for the remaining part of the investigation, since replication was better for it than for the 0.5% suspension.

The effect of age of the crumb suspension on values is shown below. Readings were taken at 15-minute intervals on suspensions 5 to 90 minutes old.

Bread	Crumb Suspension Age	Sedimentation Rate
	min.	%
Laboratory-baked bread 12 hours old	5	78.5
	15	78.2
	30	77.0
	45	76.7
	90	76.6

The crumb did not reach equilibrium with water until 30 minutes had elapsed and remained constant thereafter. All future determinations were made 30 minutes after preparation of the suspension.

Effect of Age, Baking Formula, and Adjuncts on Sedimentation Rates. Two fermentation times and three baking formulas were used

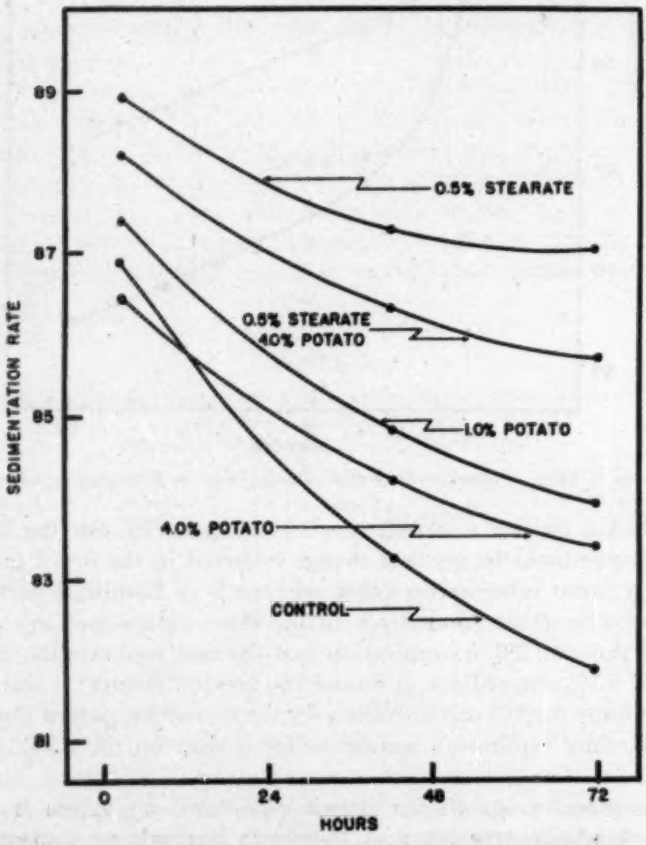

Fig .4. Average influence of adjuncts in the baking formula on crumb sedimentation rate with age of bread made with three formulas.

to determine the effect of potato flour and stearate on sedimentation values as the bread crumb aged. The results are shown in Fig. 4, each point representing an average for three baking formulas. Although Harris, Sibbitt, and Banasik (4) had found that cooked potato flour at 1% and 4% concentrations increased whereas stearate lowered crumb hydration as compared to the control, the sedimentation values were all increased by the adjuncts. Also, a comparison of the slope of the control curve with the others (Fig. 4) indicated they had a slower rate of change. Table III shows that the three sources of variation all

TABLE III

ANALYSIS OF VARIANCE OF SEDIMENTATION DATA

Source of Variation	Degrees of Freedom	Variance
Between adjuncts	4	18.9**
Between formulas	2	25.8**
Between ages	2	43.9**
Interactions:		
Adjuncts × formulas	8	1.0
Adjuncts × ages	8	0.9
Formulas × ages	4	1.4
Adjuncts × formulas × ages	16	0.5
(error)		
Total	44	

had a significant influence on sedimentation, age having the greatest effect. None of the interactions were significant.

Correlation Coefficients Between Crumb Moisture, Firmness, and Hydration. The correlation coefficients, based on data obtained on 45 samples included in this study, were as follows:

Crumb moisture × firmness	−0.03
Crumb moisture × hydration	+0.75**
Crumb moisture × sedimentation	+0.53**
Firmness × hydration	−0.32*
Firmness × sedimentation	−0.51**
Hydration × sedimentation	+0.55**

*Significant at 5% point
**Significant at 1% point

The dependence of hydration and sedimentation on crumb moisture is shown by the highly significant correlations. Firmness was posi-

tively correlated with sedimentation. Apparently crumb moisture influenced this relation, because the partial correlation coefficient (firmness × sedimentation, with moisture held constant) was — 0.93. Thus, if transfer of moisture from the inner to the outer crumb and crust were eliminated, sedimentation rate would be closely related to crumb firmness.

Discussion

The method for measuring changes in the density of the crumb revealed differences in sedimentation rate due to crumb age, baking formula, and adjuncts. Although the relations between crumb firmness, hydration, and sedimentation were significant, none of them were sufficiently large to permit a prediction of the results of other methods from any one. A highly significant relationship (— 0.93) was obtained between firmness and sedimentation when moisture was held constant.

The results of this study give added support to the statement of Bice and Geddes (1) that any one method can measure only a few physico-chemical changes in the aging of bread. Particle aggregation and density of the crumb were affected by moisture transfer as the bread aged, as well as by method of crumb preparation.

Literature Cited

1. Bice, C. W., and Geddes, W. F. Studies on bread staling. IV. Evaluation of methods for the measurement of changes which occur during bread staling. Cereal Chem. **26:** 440–465 (1949).
2. Cathcart, W. H., and Luber, S. V. Modification of the "swelling power" test for the staling of bread. Cereal Chem. **16:** 430–440 (1939).
3. Harris, R. H., and Jesperson, Ethel. A study of the effect of various factors on the swelling of certain cereal starches. J. Colloid Sci. **1:** 479–493 (1946).
4. Harris, R. H., Sibbitt, L. D., and Banasik, O. J. Effect of potato flour on bread quality and changes with age. Cereal Chem. **29:** 123–131 (1952).

COOKIE FLOUR STUDIES. I. STREAM ANALYSIS BY MEANS OF THE COOKIE TEST[1]

G. F. GARNATZ[2], W. H. HANSON[3], AND R. C. LAKAMP[2]

ABSTRACT

The spreading factor of cookies baked from various mill streams obtained in milling soft white winter wheat decreased from the head to the tail of the mill and also within each group of flours. The break streams yielded flours of superior spreading value to the middlings streams. In general, as the spread factor diminished, the protein, ash, and apparent viscosity of the flour increased. These trends were uniformly observed in four lots of mill streams representing two wheat types and two crop years. Cookies made from various mill streams showed the same trends in quality when baked with or without the inclusion of ammonium bicarbonate in the baking formula.

An improved cookie flour was obtained by stream selection after modifying a soft wheat mill to obtain a greater release of stock at the break rolls.

Soft wheat milling techniques might be advantageously reviewed to determine whether revisions in the flow and balance of the mill may lead to the production of improved cookie flours.

For some time, apparent viscosity has been employed as an index of the quality of flour for making cookies. Although it is a useful guide, shop experience does not always confirm the indications obtained from this test. In the authors' experience, the spreading property of a flour obtained from the A.A.C.C. cookie-baking test (1) is a more dependable index of shop performance than apparent viscosity. However, the use of both criteria permits a better evaluation than either one alone.

An instance has been encountered where two mills drawing wheat from the same general area produced flours of different cookie-making quality, and the present investigation was undertaken to determine whether this could be attributed to differences in milling. The experiments were designed to secure information on the effect of several variables on cookie-making quality, including the nature of the wheat, the crop year, and the milling technique. The effect of storage on the cookie-making value of flours was also investigated. This involved comparison of cookie-baking tests made several months apart, and experiments were carried out to determine whether it was necessary to include ammonium bicarbonate in the cookie formula. This chemical

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[2] Kroger Food Foundation, Cincinnati, Ohio.
[3] F. W. Stock and Sons, Inc., Hillsdale, Michigan.

acts so rapidly that it might well be an important source of variability in the cookie test. Finney, Yamazaki, and Morris (2) have reported that while the concentration of ammonium bicarbonate used in the formula is not critical in routine work, it does influence the diameter and spread factor of cookies baked from flours milled from a given wheat variety.

Materials and Methods

Seven sets of flours and mill streams were obtained as follows:

1. Mill A (Michigan); early portion 1949 crop.
2. Mill A (Michigan); late portion 1949 crop.
3. Mill A (Michigan); 1950 crop.
4. Mill B (Michigan); 1949 crop.
5. Mill B (Michigan); 1950 crop.
6. Mill C (Washington); 1949 crop.
7. Mill C (Washington); 1950 crop.

In addition to the determination of moisture, protein, and ash by the usual methods, the apparent viscosity of each flour was determined by the method described in Cereal Laboratory Methods (1), except that the digestion period was omitted. The maximum viscosity value, regardless of the increment of acid employed, was recorded as the apparent viscosity. Samples collected from Mill A during the early part of the 1949 crop were baked by the A.A.C.C. cookie test described by Hanson (3). These flours were stored for 8 months at 5°C., after which

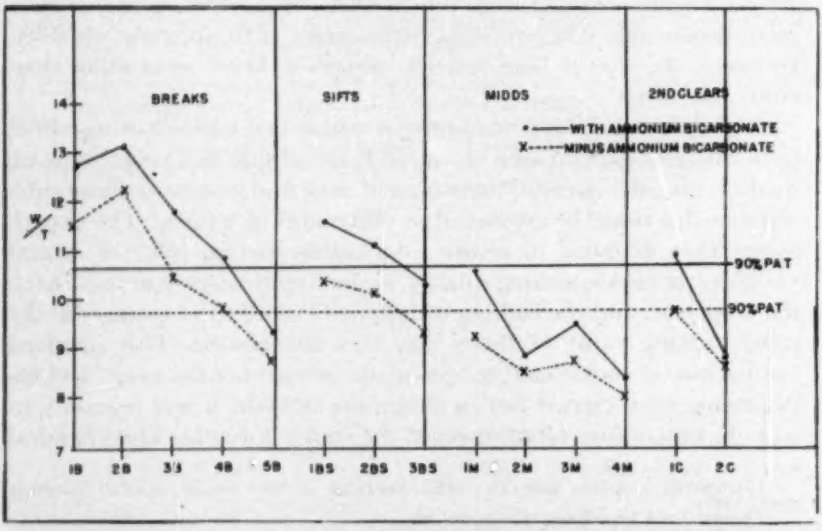

Fig. 1. Relation between the spread factor (W/T) of cookies made with and without ammonium bicarbonate. The flours were produced in Michigan mill A from the early part of the 1949 crop and were baked after storage for 8 months at 5°C.

apparent viscosity and cookie tests were again made. Where sufficient sample was available, cookies were made both with and without ammonium bicarbonate in the baking formula. In the cookie-baking tests for the other series of samples, ammonium bicarbonate was omitted. Photographs were made of the cookies to show their top grain and relative diameters; the spread factor, W/T, was also determined, where W is the diameter and T the thickness.

Results

Effect of Ammonium Bicarbonate on the Spreading Factor. The spreading factors for cookies made with and without ammonium bicarbonate on several streams taken from mill A and baked after storage

TABLE I

ANALYTICAL DATA FOR STREAMS OBTAINED FROM MICHIGAN MILL (A) FROM THE EARLY PART OF THE 1949 CROP

Flour	Percent of Total Flour	Protein[1]	Ash[1]	Apparent Viscosity	W/T
		%	%		
90% Patent		7.8	0.36	27	9.6
10% Clear		9.2	0.72	23	7.5
First break	3	5.2	0.26	15	11.3
Second break	6	5.6	0.28	18	11.3
Third break	6	6.9	0.31	34	10.3
Fourth break	4	7.8	0.44	34	9.3
Fifth break	3	8.8	0.64	20	8.9
Break sifts No. 1	8	6.9	0.33	30	9.9
Break sifts No. 2		7.0	0.34	35	9.7
Break sifts No. 3		8.4	0.36	58	9.5
Sizings	4	6.2	0.33	22	9.9
First middlings	15	7.0	0.29	36	9.5
Second middlings	15	8.0	0.30	65	9.0
Third middlings	10	8.3	0.35	58	8.6
Fourth middlings	4	9.4	0.33	68	8.6
Fifth middlings	4	9.5	0.49	46	7.7
First tailings	3	6.8	0.45	19	9.7
Second tailings	3	8.1	0.71	23	8.2
First low grade	4	10.0	0.58	39	7.0
Second low grade	3	10.2	0.72	19	6.6
Feed reel		10.8	0.96	33	5.7
Finishing reel		10.6	0.81	19	6.9
Third clear reel		9.4	0.89	16	7.5
B. S. duster	5	9.2	0.88	14	8.2

[1] Values expressed on a 14.0% moisture basis.

for 8 months at 5°C. are shown in Fig. 1. The correlation between the values for corresponding mill streams is excellent, and led to the omission of this chemical from the formula used in testing all samples except those which were taken from mill A for the early portion of the 1949 crop.

Cookie-Making Quality of Different Streams. The results obtained for the mill-stream samples from the Michigan mill, A, obtained from the early part of the 1949 crop, are recorded in Table I. The corresponding data for samples obtained from this same mill from the late portion of the 1949 crop are shown graphically in Fig. 2.

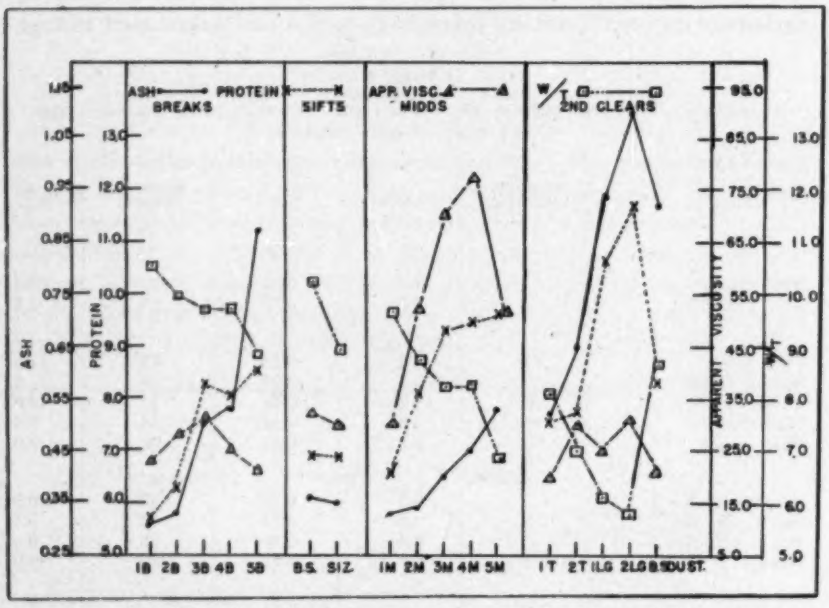

Fig. 2. Interrelationships between protein, ash, apparent viscosity, and spread factor (W/T) for various flour streams from Michigan mill A utilizing soft white winter wheat from the late portion of the 1949 crop

The spread factor decreases, in general, from the head to the tail of the mill; it also decreases progressively within each section of the mill. The break streams yield flours of superior spreading value to the middlings streams. In general, as the spread factor diminished, the protein, ash, and apparent viscosity increased. Both the quantity and quality of the flour proteins seem to influence the spread factor, as the relation between apparent viscosity and cookie quality is not particularly good.

Photographs of cookies made from the flours listed in Table I are

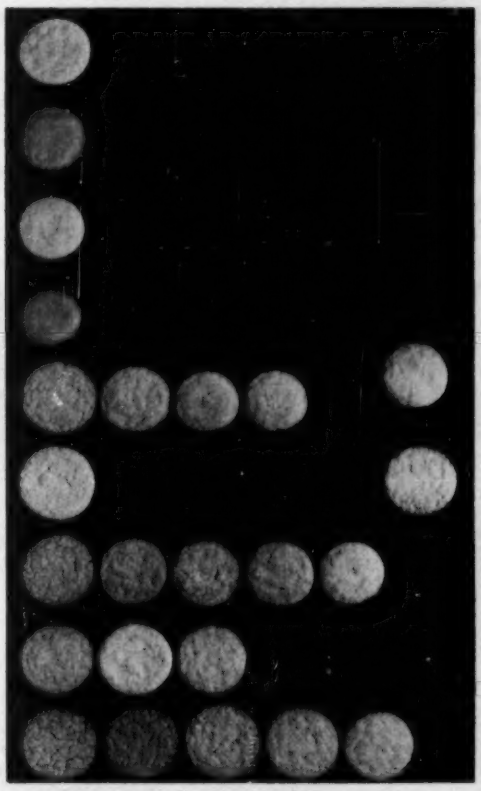

Fig. 3. Cookie characteristics of the various streams from mill A, utilizing soft white winter wheat from the early part of the 1949 crop. Reading from bottom to top and left to right, the cookies represent the following streams:

Row	
1	Break streams 1, 2, 3, 4, and 5
2	Break sifts 1, 2, and 3
3	Middlings streams 1, 2, 3, 4, and 5
4	Sizings; 90% patent (all breaks, first four midds, all break sifts, sizings, both tailings and first low grade)
5	First and second tailings; first and second low grade; 10% clear (fifth midds, second low grade, finishing reel, bran and shorts duster, third clear reel, feed reel)
6	Feed reel
7	Finishing reel
8	Third clear reel
9	Bran and shorts duster

reproduced in Fig. 3. Cookies made from break streams diminish in diameter, lose the characteristic cracked appearance on the top, and become smoother in progressing from the first to the fifth break. The flours from the break sift reels yield cookies of good spreading properties; they differ less than would be expected from their viscosity values. The five middlings streams spread less than the break and break sift flours and, after the second middlings, this value drops sharply and

the cookies develop rather smooth tops which indicate "hardness." The sizings and first tailings flours produced good cookies but the second tailings flour, the two low-grade streams, feed reel, finishing reel, and third clear reel flours all gave poor cookies. The spreading properties of flour from the bran and shorts duster were no better than those for such streams as the third middlings and second tailings flours, although the apparent viscosity is comparable to that for the first and second break flours.

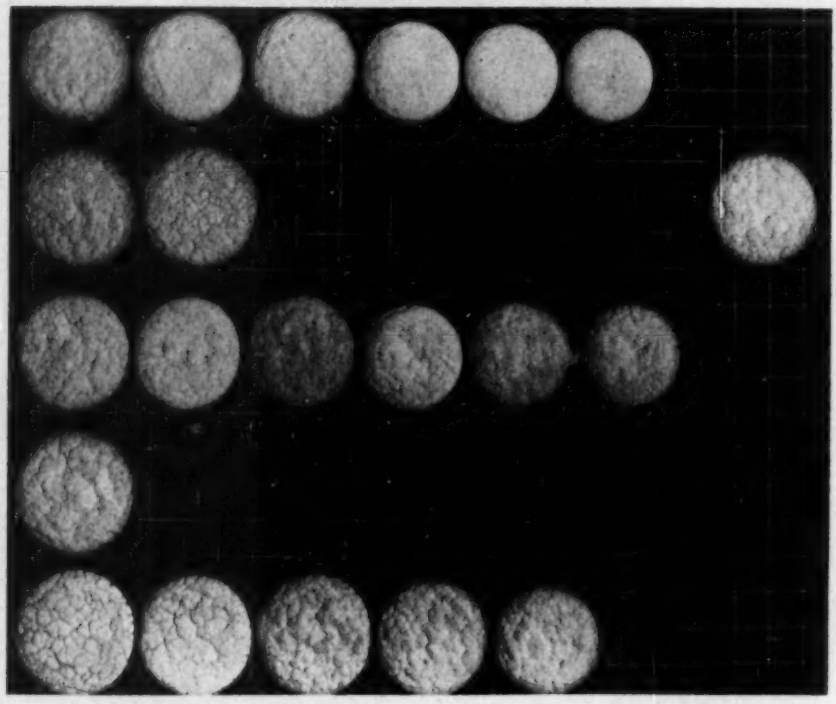

Fig. 4. Cookie characteristics of the various streams from Michigan mill B utilizing soft white winter wheat from the 1949 crop. Reading from bottom to top and left to right, the cookies represent the following streams:

Row
1 Break streams 1, 2, 3, 4, and 5
2 Break cuts
3 Middlings streams 1, 2, 3, 4, 5, and 6
4 Quality and sizings streams; patent flour
5 Tailings 1, 2, and 3; low grade 1, 2, and 3

When the samples from mill A taken from the early part of the 1949 crop were rebaked after storage for 8 months at 5°C., the apparent viscosities were essentially unchanged, whereas the spreading properties appeared to show some improvement.

The cookie tests on samples from Michigan mill A taken from the

late part of the 1949 crop revealed the same trends in cookie quality from the head to the tail of the mill as were shown by the earlier samples. This was also true for the samples from Michigan mill B, as can be seen from Fig. 4. The same generalizations can be drawn from the photographs in Fig. 5 showing cookies baked from various streams taken from mill C in Washington which was operating on an entirely different type of soft wheat from that of the two mills in Michigan. There is a gradual loss in spreading property in progressing from the splitter break, through the five breaks. The middlings streams do not spread as well as the break streams; indeed the variation shown by

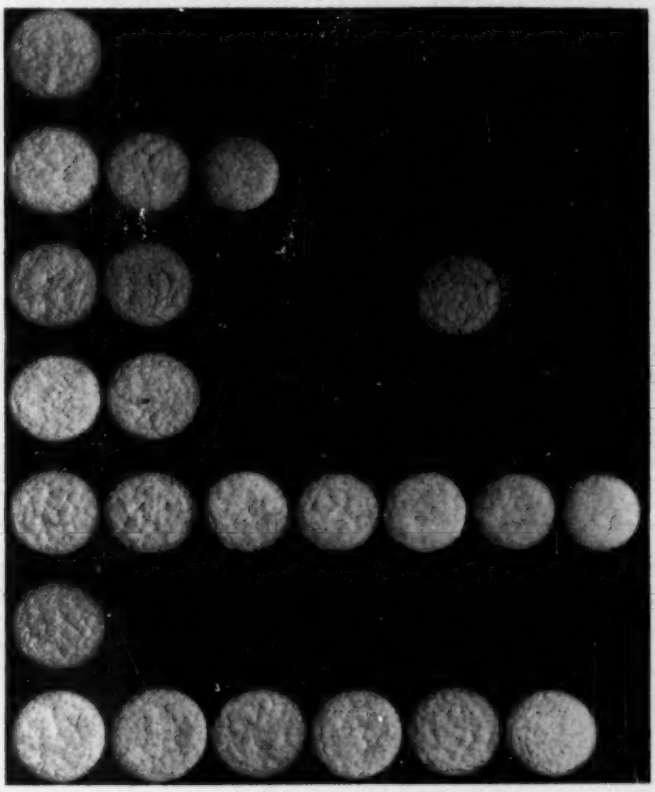

Fig. 5. Cookie characteristics of the various streams from Washington mill C, utilizing soft wheat from the 1949 crop. Reading from bottom to top and left to right, the cookies represent the following streams:

Row
1 Splitter break and the break streams 1 to 5 inclusive.
2 Break redust
3 Middlings 1 to 7 inclusive
4 Quality and sizings
5 Good and poor suction; 100% flour
6 Tailings 1 and 2; low grade
7 Bran and shorts duster

the different middlings flours covers the full range of spread and top grain encountered in the streams from this mill. The quality, sizings, good and poor suction, and first tailings streams yielded fairly satisfactory cookies, but the quality of the cookies from the second tailings and low grade flours was poor. The cookies from the bran and shorts duster flour had desirable properties.

The relations between the spread factor and the various streams obtained from mills A, B, and C in the 1950 crop are shown in Fig. 6.

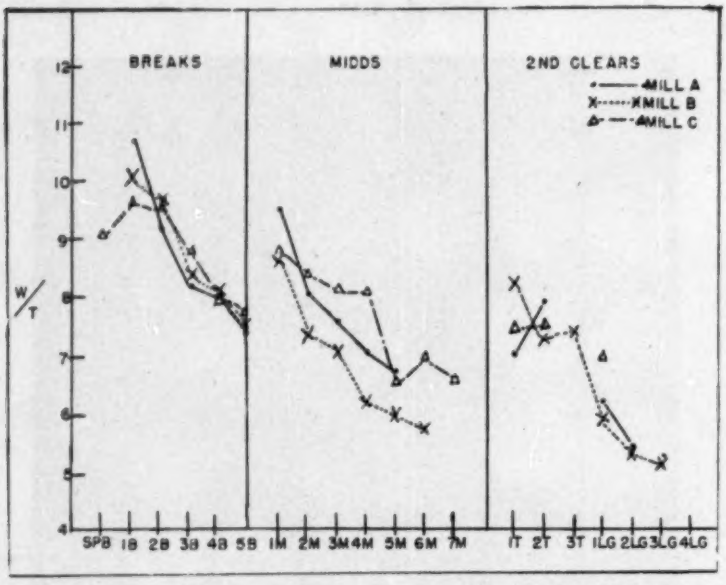

Fig. 6. Comparison of the spread factor (W/T) for cookies baked from various streams obtained from three mills. Mills A and B were located in Michigan and mill C in Washington.

The streams are arranged in proper progression into various groups and the graph clearly shows how the spread factor diminishes within each group and from head to tail of the mill.

Discussion

These results clearly show that the break streams make better cookies than the middlings streams. With this information at hand, a mill was modified to obtain a greater release of stock at the break rolls and, consequently, a greater yield of break flour. This modification, coupled with stream selection, yielded an improved cookie flour.

Soft wheat milling techniques might well be reviewed to determine

whether the tradition inherited from hard wheat milling, – namely, to produce a maximum of middlings stock – might not be revised advantageously in the direction of endeavoring to secure a maximum yield of break stocks. Other revisions of the flow and in the balance of the mill may lead to the production of improved cookie flours.

Acknowledgment

The authors express their thanks to J. W. Montzheimer, Centennial Flouring Mills Co., Spokane, Washington; J. W. Elling, International Milling Co., Detroit, Michigan; and F. W. Stock and Sons, Inc., Hillsdale, Michigan, for the samples of mill streams which made this study possible. They also gratefully acknowledge the helpful advice of R. Sopher, Garland Mills, Inc., Greensburg, Indiana, on modifications in milling procedure.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.), Am. Assoc. Cereal Chem.: St. Paul 1, Minn. (1947).
2. Finney, K. F., Yamazaki, W. T., and Morris, V. H. Effects of varying quantities of sugar, shortening, and ammonium bicarbonate on the spreading and top grain of sugar-snap cookies. Cereal Chem. **27**: 30–41 (1950).
3. Hanson, W. H. Report of the 1941–42 committee on testing biscuit and cracker flours. Cereal Chem. **20**: 314–318 (1943).

BOOK REVIEW

Die Ernährung, —Physiologie, Pathologie, Therapie. By Konrad Lang and Rudolph Schoen. VII + 624 pp. Springer-Verlag, Berlin, Göttingen, Heidelberg, 1952. Price: DM 58.

This book appeared shortly after the publication of "Stoffwechsel and Ernährung" by Lang and Ranke (1950) and "Der Intermediäre Stoffwechsel" by Lang (1952). Springer, the publishers, and particularly Dr. Lang have performed a real service in making available in the German language these summaries of current knowledge in the broad field of nutrition.

In the compilation of this book on human nutrition, Lang and Schoen have had the collaboration of specialists in various fields who have contributed one or more chapters, such as Lehnartz (Chemistry of Foods, 60 pp.); Diemair (Preparation of Foods, 58 pp.); Mellinghoff (Dietetics, 56 pp.); Nitschke (Physiology and Pathology of Infant Nutrition, 31 pp.) and Rickets, Tetany and Osteomalacia (15 pp.); Kühnau (Physiology of the Vitamins, 51 pp.); Jürgens (Clinical Symptomatology and Therapy of Avitaminosis-A, 44 pp. and Symptomatology and Therapy of Avitaminosis-E, 20 pp.); Vanotti (The B-vitamins, 45 pp.); Fähndrich (Clinical Manifestations and Therapy of Diseases of Vitamin C Deficiency, 28 pp.). Short chapters by Dean (Cambridge) on guidance of peoples' nutrition and by Koller on Vitamin K are also included. The editors have themselves contributed excellent chapters on Physiology of Nutrition (68 pp.) by Lang and on Undernutrition, Malnutrition, and Overnutrition (77 pp.) by Schoen.

The advantages of authoritative presentation of the subject matter are somewhat marred, in a book of this type, by a lack of uniformity of presentation and particularly of documentation of statements made through summary tables, charts and bibliographies. In some instances the bibliography is extensive and reflects the worldwide literature, in others it is restricted merely to the German papers. Following the important subject on the physiology of the vitamins, only six references are cited while with that on the B-vitamins no bibliography is given, although in the text names of investigators are referred to parenthetically.

The chapter by Schoen on undernutrition, malnutrition, and overnutrition though somewhat unbalanced, is very interesting; it deals primarily with the effects of chronic undernutrition while malnutrition (i.e., dietary imbalance) and overnutrition are relegated to a briefer treatment than they perhaps deserve. Schoen correctly points out the great limitations of the concept of "normal values" which are so often used to assess the clinical status of an individual without sufficient regard to the large deviations from "normal," which, for the individual concerned, may not be at all abnormal. One might question the view that "the description of undernutrition is no longer a problem of current concern," and it is only partly true that "a period of hunger can now also be evaluated finally with respect to its belated and permanent damage" (p. 204). The methodology for such assessment is not sufficiently well defined and precise; the recognition and availability of supposedly "normal control subjects" as a basis for comparison are problems which can be solved only through well organized, subsidized and long-term studies. It is to be hoped that a wide dissemination of this book by Lang and Schoen, particularly in the medical profession will lead to a greater awareness among clinicians and those concerned with public health that there exists in the present population, particularly in Europe, a large reservoir of people in whom the chronic effects of temporary undernutrition and malnutrition should be studied throughout their lives and postmortem.

The printing, paper and make-up of the book are excellent. For those who are well versed in German "Die Ernährung" is a most valuable addition to a library; for others, particularly advanced students who ought to improve their understanding of German, this book would provide valuable information and training at the same time.

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